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(54) **Title:** NUCLEIC ACIDS AND PROTEINS FROM GROUP B STREPTOCOCCUS

(57) **Abstract**

Novel protein antigens from Group B *Streptococcus* are described, together with nucleic acid sequences encoding them. Their use in vaccines and screening methods is also described.

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NUCLEIC ACIDS AND PROTEINS FROM GROUP B STREPTOCOCCUS

The present invention relates to proteins derived from *Streptococcus agalactiae*, nucleic acid molecules encoding such proteins, and the use of the proteins as antigens and/or immunogens and in detection/diagnosis. It also relates to a method for the rapid screening of bacterial genomes to isolate and characterise bacterial cell envelope associated or secreted proteins.

The *Group B Streptococcus* (GBS) *(Streptococcus agalactiae)* is an encapsulated bacterium which emerged in the1970s as a major pathogen of humans causing sepsis and meningitis in neonates as well as adults. The incidence of early onset neonatal infection during the first 5 days of life varies from 0.7 to 3.7 per 1000 live births and causes mortality in about 20% of cases. Between 25-50% of neonates surviving early onset infections frequently suffer neurological sequalae. Late onset neonatal infections occur from 6 days to three months of age at a rate of about 0.5 - 1.0 per 1000 live births.

There is an established association between the colonisation of the maternal genetic tract by GBS at the time of birth and the risk of neonatal sepsis. In humans it has been established that the rectum may act as a reservoir for GBS. Susceptibility in the neonate is correlated with the a low concentration or absence of IgG antibodies to the capsular polysaccharides found on GBS causing human disease. In the USA strains isolated from clinical cases usually belong to capsular serotypes Ia, Ib, II, III although serotype V may be of increasing significance. Type VIII GBS is the major cause of neonatal sepsis in Japan.

A possible means of prevention involves intra or postpartum administration of antibiotics to the mother but there are concerns that this might lead to the emergence of resistant organisms and in some cases allergic reactions. Vaccination of the adolescent females to induce long lasting maternally derived immunity is one of the most promising approaches to prevent GBS infections in neonates. The capsular

polysaccharide antigens of these organisms have attracted most attention as with regard to vaccine development. Studies in healthy adult volunteers have shown that serotype 1a, II and III polysaccharides are non-toxic and immunogenic in approximately 65%, 95% and 70% of non-immune adults respectively. One of the problems with using capsule antigens as vaccines is that the response rates vary according to pre-immunisation status and the polysaccharide antigen and not all vaccinees produce adequate levels of IgG antibody as indicated in vaccination studies with GBS polysaccharides in human volunteers.

Some people do not respond despite repeated stimuli. These properties are due to the T-independent nature of polysaccharide antigens. One strategy to enhance the immunogenicity of these vaccines is to enhance the T cell dependent properties of polysaccharides by conjugating them to a protein. The use of polysaccharide conjugates looks promising but there are still unresolved questions concerning the nature of the carrier protein. A conjugate vaccine against GBS would require at least 4 different conjugates to be prepared adding to the cost of a vaccine.

Recent evidence also suggests that bacterial surface proteins may be useful to confer immunity. A protein called Rib which is found on most serotype III strains but rarely on serotypes Ia, Ib or II confers immunity to challenge with Rib expressing GBS in animal models (Stalhammar-Carlemalm *et al., Journal of Experimental Medicine* **177**:1593-1603 (1993)). Another surface protein of interest as a component of a vaccine is the alpha antigen of the C proteins which protected vaccinated mice against lethal infection with strains expressing alpha protein. The amount of antigen expressed by GBS strains varies markedly.

Approaches to vaccination against GBS infections which rely on the use of capsular polysaccharides have the disadvantage that response rates are likely to vary considerably according to pre-immunisation status and the particular type of polysaccharide antigen used. Results of trials in human volunteers have indicated that

response rates may only be around 65% for some of the key capsule antigens (Larsson *et al.*, *Infection and Immunity* **64**:3518-3523 (1996)). It is also not clear whether all individuals responding to the vaccine would have adequate levels of polysaccharide specific IgG which can cross the placenta and afford immunity to neonates. By conjugating a protein carrier to the polysaccharide antigen it may be possible to convert them to T-cell dependent antigens and enhance their immunogenicity.

Preliminary studies with GBS type III polysaccharide-tetanus toxoid conjugate have been encouraging (Baker *et al.*, *Reviews of Infectious Diseases* **7**:458-467 (1985), Baker *et al.*, *The New England Journal of Medicine* **319**:1180-1185 (1988), Paoletti *et al.*, *Infection and Immunity* **64**:677-679 (1996), Paoletti *et al.*, *Infection and Immunity* **62**:3236-3243 (1994)) but in developed countries the use of tetanus may be disadvantageous since most adults will have been immunised against tetanus within the past five years. Additional boosters with tetanus toxoid may cause adverse reactions (Boyer., *Current Opinions in Pediatrics* **7**:13-18 (1995)). The polysaccharide conjugate vaccines have the disadvantage of being costly to produce and manufacture in comparison with many other kinds of vaccines. There is also the possible risk of problems caused by the cross reactivity between GBS polysaccharides and sialic acid-containing human glycoproteins.

An alternative to polysaccharides as antigens is the use of protein antigens derived from GBS. Recent evidence suggest that the GBS surface associated proteins Rib and alpha C protein may be used to confer immunity to GBS infections in experimental model systems (Stalhammar-Carlemalm *et al.*, (1993) [*supra*], Larsson *et al.*, (1996) [*supra*]). However these two proteins are not conserved in all serotypes of GBS which cause disease in humans. Assuming that these antigens would be immunogenic and elicit protective level responses in humans they would not confer protection against all infections as 10% of infectious G*roup B streptococci* do not express Rib or C protein alpha.

This invention seeks to overcome the problem of vaccination against GBS by using a novel screening method specifically designed to identify those *Group B Streptococcus* genes encoding bacterial cell surface associated or secreted proteins (antigens). The proteins expressed by these genes may be immunogenic, and therefore may be useful in the prevention and treatment of *Group B Streptococcus* infection. For the purposes of this application, the term immunogenic means that these proteins will elicit a protective immune response within a subject. Using this novel screening method a number of genes encoding novel *Group B Streptococcus* proteins have been identified.

Thus in a first aspect, the present invention provides a *Group B Streptococcus* protein, having a sequence selected from those shown in figure 1, or fragments or derivatives thereof.

It will be apparent to the skilled person that proteins and polypeptides included within this group may be cell surface receptors, adhesion molecules, transport proteins, membrane structural proteins, and/or signalling molecules.

Alterations in the amino acid sequence of a protein can occur which do not affect the function of a protein. These include amino acid deletions, insertions and substitutions and can result from alternative splicing and/or the presence of multiple translation start sites and stop sites. Polymorphisms may arise as a result of the infidelity of the translation process. Thus changes in amino acid sequence may be tolerated which do not affect the proteins function.

Thus, the present invention includes derivatives or variants of the proteins, polypeptides, and peptides of the present invention which show at least 50% identity to the proteins, polypeptides and peptides described herein. Preferably the degree of sequence identity is at least 60% and preferably it is above 75%. More preferably still is it above 80%, 90% or even 95%.

The term identity can be used to describe the similarity between two polypeptide sequences. A software package well known in the art for carrying out this procedure is the CLUSTAL program. It compares the amino acid sequences of two polypeptides and finds the optimal alignment by inserting spaces in either sequence as appropriate. The amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment can also be calculated using a software package such as BLASTx. This program aligns the largest stretch of similar sequence and assigns a value to the fit. For any one pattern comparison several regions of similarity may be found, each having a different score. One skilled in the art will appreciate that two polypeptides of different lengths may be compared over the entire length of the longer fragment. Alternatively small regions may be compared. Normally sequences of the same length are compared for a useful comparison to be made.

Manipulation of the DNA encoding the protein is a particularly powerful technique for both modifying proteins and for generating large quantities of protein for purification purposes. This may involve the use of PCR techniques to amplify a desired nucleic acid sequence. Thus the sequence data provided herein can be used to design primers for use in PCR so that a desired sequence can be targeted and then amplified to a high degree.

Typically primers will be at least five nucleotides long and will generally be at least ten nucleotides long (e.g. fifteen to twenty-five nucleotides long). In some cases primers of at least thirty or at least thirty-five nucleotides in length may be used.

As a further alternative chemical synthesis may be used. This may be automated. Relatively short sequences may be chemically synthesised and ligated together to provide a longer sequence.

Thus in a further aspect, the present invention provides , a nucleic acid molecule comprising or consisting of a sequence which is:

(i) any of the DNA sequences set out in figure 1 herein or their RNA equivalents;

(ii) a sequence which is complementary to any of the sequences of (i);

(iii) a sequence which codes for the same protein or polypeptide, as those sequences of (i) or (ii);

(iv) a sequence which is shows substantial identity with any of those of (i), (ii) and (iii); or

(v) a sequence which codes for a derivative or fragment of a nucleic acid molecule shown in figure 1.

The term identity can also be used to describe the similarity between two individual DNA sequences. The 'bestfit' program (Smith and Waterman, Advances in applied Mathematics, 482-489 (1981)) is one example of a type of computer software used to find the best segment of similarity between two nucleic acid sequences, whilst the GAP program enables sequences to be aligned along their whole length and finds the optimal alignment by inserting spaces in either sequence as appropriate.

The term 'RNA equivalent' when used above indicates that a given RNA molecule has a sequence which is complementary to that of a given DNA molecule, allowing for the fact that in RNA 'U' replaces 'T' in the genetic code. The nucleic acid molecule may be in isolated or recombinant form.

The nucleic acid molecule may be in an isolated or recombinant form. DNA constructs can readily be generated using methods well known in the art. These techniques are disclosed, for example in J. Sambrook *et al*, *Molecular Cloning 2nd Edition*, Cold Spring Harbour Laboratory Press (1989). Modifications of DNA constructs and the proteins expressed such as the addition of promoters, enhancers, signal sequences, leader sequences, translation start and stop signals and DNA stability controlling regions, or the addition of fusion partners may then be facilitated.

Normally the DNA construct will be inserted into a vector which may be of phage or plasmid origin. Expression of the protein is achieved by the transformation or transfection of the vector into a host cell which may be of eukaryotic or prokaryotic origin. Such vectors and suitable host cells form yet further aspects of the present invention.

The *Group B Streptococcus* proteins (antigens) described herein can additionally be used to raise antibodies, or to generate affibodies. These can be used to detect *Group B Streptococcus.*

Thus in a further aspect the present invention provides, an antibody, affibody, or a derivative thereof which binds to any one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, as described herein.

Antibodies within the scope of the present invention may be monoclonal or polyclonal. Polyclonal antibodies can be raised by stimulating their production in a suitable animal host (e.g. a mouse, rat, guinea pig, rabbit, sheep, goat or monkey) when a protein as described herein, or a homologue, derivative or fragment thereof, is injected into the animal. If desired, an adjuvant may be administered together with the protein. Well-known adjuvants include Freund's adjuvant (complete and incomplete) and aluminium hydroxide. The antibodies can then be purified by virtue of their binding to a protein as described herein.

Monoclonal antibodies can be produced from hybridomas. These can be formed by fusing myeloma cells and spleen cells which produce the desired antibody in order to form an immortal cell line. Thus the well-known Kohler & Milstein technique (*Nature* **256** (1975)) or subsequent variations upon this technique can be used.

Techniques for producing monoclonal and polyclonal antibodies that bind to a particular polypeptide/protein are now well developed in the art. They are discussed in standard

immunology textbooks, for example in Roitt *et al*, *Immunology* second edition (1989), Churchill Livingstone, London.

In addition to whole antibodies, the present invention includes derivatives thereof which are capable of binding to proteins etc as described herein. Thus the present invention includes antibody fragments and synthetic constructs. Examples of antibody fragments and synthetic constructs are given by Dougall *et al* in *Tibtech* **12** 372-379 (September 1994).

Antibody fragments include, for example, Fab, $F(ab')_2$ and Fv fragments. Fab fragments (These are discussed in Roitt *et al* [*supra*]). Fv fragments can be modified to produce a synthetic construct known as a single chain Fv (scFv) molecule. This includes a peptide linker covalently joining V_h and V_l regions, which contributes to the stability of the molecule. Other synthetic constructs that can be used include CDR peptides. These are synthetic peptides comprising antigen-binding determinants. Peptide mimetics may also be used. These molecules are usually conformationally restricted organic rings that mimic the structure of a CDR loop and that include antigen-interactive side chains.

Synthetic constructs include chimaeric molecules. Thus, for example, humanised (or primatised) antibodies or derivatives thereof are within the scope of the present invention. An example of a humanised antibody is an antibody having human framework regions, but rodent hypervariable regions. Ways of producing chimaeric antibodies are discussed for example by Morrison *et al* in *PNAS*, **81,** 6851-6855 (1984) and by Takeda *et al* in *Nature*. **314,** 452-454 (1985).

Synthetic constructs also include molecules comprising an additional moiety that provides the molecule with some desirable property in addition to antigen binding. For example the moiety may be a label (e.g. a fluorescent or radioactive label). Alternatively, it may be a pharmaceutically active agent.

Affibodies are proteins which are found to bind to target proteins with a low dissociation constant. They are selected from phage display libraries expressing a segment of the target protein of interest (Nord K, Gunneriusson E, Ringdahl J, Stahl S, Uhlen M, Nygren PA, Department of Biochemistry and Biotechology, Royal Institute of Technology (KTH), Stockholm, Sweden).

In a further aspect the invention provides an immunogenic composition comprising one or more proteins, polypeptides, peptides, fragments or derivatives thereof, or nucleotide sequences described herein. A composition of this sort may be useful in the treatment or prevention of *Group B Streptococcus* infection in subject. In a preferred aspect of the invention the immunogenic composition is a vaccine.

In other aspects the invention provides:

i) Use of an immunogenic composition as described herein in the preparation of a medicament for the treatment or prophylaxis of *Group B Streptococcus* infection. Preferably the medicament is a vaccine.

ii) A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one antibody, affibody, or a derivative thereof, as described herein.

iii) A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one protein, polypeptide, peptide, fragments or derivatives as described herein.

iv) A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one nucleic acid molecule as described herein.

v) A kit for the detection of *Group B Streptococcus* comprising at least one antibody, affibody, or derivatives thereof, described herein.

vi) A kit for the detection of *Group B Streptococcus* comprising at least one *Group B Streptococcus* protein, polypeptide, peptide, fragment or derivative thereof, as described herein.

vii) A kit for the detection of *Group B Streptococcus* comprising at least one nucleic acid of the invention.

As described previously, the novel proteins described herein are identified and isolated using a novel screening method which specifically identifies those *Group B Streptococcus* genes encoding bacterial cell envelope associated or secreted proteins.

The information necessary for the secretion/export of proteins has been extensively studied in bacteria. In the majority of cases, export requires a signal peptide positioned at the N-terminus of the precursor protein to target the precursor to translocation sites on the membrane. During or after translocation, the signal peptide is removed by a signal peptidase. The ultimate destination/localisation of the protein, (whether it be secreted extracellularly or anchored to the bacterium's surface, etc) is determined by sequences other than the leader peptide sequence.

Recently, Poquet *et al.* (*J. Bacteriol.* **180:**1904-1912 (1998)) have described a screening vector incorporating the *nuc* gene lacking its own signal leader as a reporter to identify exported proteins in Gram positive bacteria, and have applied it to *L. lactis*. Staphylococcal nuclease is a naturally secreted heat-stable, monomeric enzyme which has been efficiently expressed and secreted in a range of Gram positive bacteria (Shortle., *Gene* **22:**181-189 (1983), Kovacevic *et al.*, *J. Bacteriol.* **162:**521-528 (1985), Miller *et al.*, *J. Bacteriol.* **169:**3508-3514 (1987), Liebl *et al.*, *J. Bacteriol.*

174:1854-1861(1992), Le Loir *et al.*, *J. Bacteriol.* **176:**5135-5139 (1994), Poquet *et al.*, 1998 [*supra*]). The screening vector (pFUN) contains the pAMβ1 replicon which functions in a broad host range of Gram-positive bacteria in addition to the ColE1 replicon that promotes replication in *Escherichia coli* and certain other Gram negative bacteria. Unique cloning sites present in the vector can be used to generate transcriptional and translational fusions between cloned genomic DNA fragments and the open reading frame of the truncated *nuc* gene devoid of its own signal secretion leader. The *nuc* gene makes an ideal reporter gene because the secretion of nuclease can readily be detected using a simple and sensitive plate test: Recombinant colonies secreting the nuclease develop a pink halo whereas control colonies remain white (Shortle, 1983 [*supra*], Le Loir *et al.*, 1994 [*supra*]).

A direct screen to identify and isolate DNA encoding bacterial cell envelope associated or secreted proteins (antigens).in pathogenic bacteria has been developed by the present inventors which utilises a vector-system (pTREP1 expression vector) in *Lactococcus lactis* that specifically detects DNA sequences which are adjacent to, and associated with DNA encoding surface proteins from *Group B Streptococcus*. The screening vector also incorporates the *nuc* gene encoding the *Staphylococcal* nuclease as a reporter gene.

Only the part of the *nuc* gene encoding the mature nuclease protein (minus its signal peptide sequence) is cloned into the pTREP1 expression vector in *L. lactis*. In this form, the *nuc*-encoded nuclease cannot be secreted even when expressed intracellularly. The reporter vector is then randomly combined with appropriately digested genomic DNA from *Group B Streptococcus*, cloned into *L. lactis* and used as a screening system for sequences permitting the export of nuclease. In this way gene/partial gene sequences encoding exported proteins from *Group B Streptococcus* are isolated. Once a partial gene sequence is obtained, full length sequences encoding exported proteins can readily be obtained using techniques well known in the art.

In possessing a promoter, the pTREP1-*nuc* vectors differ from the pFUN vector described by Poquet *et al.* (1998) [*supra*], which was used to identify *L. lactis* exported proteins by screening directly for *Nuc* activity directly in *L. lactis*. As the pFUN vector does not contain a promoter upstream of the *nuc* open reading frame the cloned genomic DNA fragment must also provide the signals for transcription in addition to those elements required for translation initiation and secretion of *Nuc*. This limitation may prevent the isolation of genes that are distant from a promoter for example genes which are within polycistronic operons. Additionally there can be no guarantee that promoters derived from other species of bacteria will be recognised and functional in *L. lactis*. Certain promoters may be under stringent regulation in the natural host but not in *L. lactis*. In contrast, the presence of the P1 promoter in the pTREP1-*nuc* series of vectors ensures that promoterless DNA fragments (or DNA fragments containing promoter sequences not active in *L. lactis*) may still be transcribed. Thus yet another advantage of this invention is that genes missed in other screening methods may be identified.

Hence in a further aspect the present invention provides a method of screening for DNA encoding bacterial cell wall associated or surface antigens in gram positive bacteria comprising the steps of:

- combining a reporter vector including the nucleotide sequence encoding the mature from of the staphylcoccus nuclease gene and an upstream promoter region with DNA from a gram positive bacteria.
- transforming the resultant vector into *Lactococcus lactis* cells.
- assaying for the secretion of *staphlycoccus* nuclease protein in the transformed cells.

Preferably, the reporter vector is one of the pTREP1-*nuc* vectors shown in figure 4.

In another aspect, the present invention provides a vector as shown in figure 4 for use in screening for DNA encoding exported or surface antigens in gram positive bacteria. Examples of gram positive bacteria which may be screened include *Group B Streptococcus*, *Streptococcus pneumoniae*, *Staphylcoccus aureus or pathogenic Group A Streptococci.*

Given that the inventors have identified a group of important proteins, such proteins are potential targets for anti-microbial therapy. It is necessary, however, to determine whether each individual protein is essential for the organism's viability. Thus, the present invention also provides a method of determining whether a protein or polypeptide as described herein represents a potential anti-microbial target which comprises inactivating said protein and determining whether *Group B Streptococcus* is still viable.

A suitable method for inactivating the protein is to effect selected gene knockouts, ie prevent expression of the protein and determine whether this results in a lethal change. Suitable methods for carrying out such gene knockouts are described in Li *et al* , *P.N.A.S.,* **94**:13251-13256 (1997) and Kolkman *et al*

In a final aspect the present invention provides the use of an agent capable of antagonising, inhibiting or otherwise interfering with the function or expression of a protein or polypeptide of the invention in the manufacture of a medicament for use in the treatment or prophylaxis of *Group B Streptococcus* infection.

The invention will now be described by means of the following example which should not in any way be construed as limiting. The examples refer to the figures in which

Fig 1: (A) Shows a number of full length nucleotide sequences encoding antigenic *Group B Streptococcus* proteins. (B) Shows the corresponding amino acid sequences.

Fig 2: Shows a number of oligonucleotide primers used in the screening process

nucS1 primer designed to amplify a mature form of the nuc A gene

nucS2- primer designed to amplify a mature form of the nuc A gene.

nucS3 primer designed to amplify a mature form of the nuc A gene

nucR primer designed to amplify a mature form of the nuc A gene

nucseq primer designed to sequence DNA cloned into the pTREP-Nuc vector

pTREPF nucleic acid sequence containing recognition site for ECORV. Used for cloning fragments into pTREX7.

pTREPR nucleic acid sequence containing recognition site for BAMH1. Used for cloning fragments into pTREX7.

PUCF forward sequencing primer, enables direct sequencing of cloned DNA fragments.

VR example of gene specific primer used to obtain further antigen DNA sequence by the method of DNA walking.

V1 example of gene specific primer used to obtain further antigen DNA sequence by the method of DNA walking.

V2 example of gene specific primer used to obtain further antigen DNA sequence by the method of DNA walking.

Fig 3: (i) Schematic presentation of the nucleotide sequence of the unique gene cloning site immediately upstream of the mature *nuc* gene in pTREP1-*nuc*1, pTREP1-*nuc*2 and pTREP1-*nuc*3. Each of the pTREP-*nuc* vectors contain an

EcoRV (a SmaI site in pTREP1-*nuc*2) cleavage site which allows cloning of genomic DNA fragments in 3 different frames with respect to the mature *nuc* gene.

(ii) A physical and genetic summary map of the pTREP1-*nuc* vectors. The expression cassette incorporating *nuc*, the macrolides, lincosamides and streptogramin B (MLS) resistance determinant, and the replicon (rep) *Ori*-pAMβ1 are depicted (not drawn to scale).

(iii) Schematic presentation of the expression cassette showing the various sequence elements involved in gene expression and location of unique restriction endonuclease sites (not drawn to scale).

Fig 4: Shows the results of various DNA vaccine trials;

Fig 5: Shows the results of a second group of DNA vaccine trials;

Figs 6-11: Show various Southern Blot analyses of different Group B streptococcus strains.

Example 1

Thus far more than 100 gene/partial gene sequences putatively encoding exported proteins in *S. agalactiae* have been identified using the nuclease screening system of the invention. These have been further analysed to remove artifacts. The nucleotide sequences of genes identified using the screening system has been characterised using a number of parameters described below. All of these sequences are novel in that they have not been described previously.

1. All putative surface proteins are analysed for leader/signal peptide sequences. Bacterial signal peptide sequences share a common design. They are characterised by a short positively charged N-terminus (N region) immediately preceding a stretch of hydrophobic residues (central portion-h region) followed by a

more polar C-terminal portion which contains the cleavage site (c-region). Computer software is used to perform hydropathy profiling of putative proteins (Marcks, *Nuc. Acid. Res.*, **16**:1829-1836 (1988)) which is used to identify the distinctive hydrophobic portion (h-region) typical of leader peptide sequences. In addition, the presence/absence of a potential ribosomal binding site (Shine-Dalgarno sequence required for translation) is also noted.

2. All putative surface protein sequences are used to search the OWL sequence database which includes a translation of the GENBANK and SWISSPROT database.. This allows identification of similar sequences which may have been previously characterised not only at the sequence level but at a functional level. It may also provide information indicating that these proteins are indeed surface related and not artifacts.

3. Putative *S. agalactiae* surface proteins are also be assessed for their novelty. Some of the identified proteins may or may not possess a typical leader peptide sequence and may not show homology with any DNA/protein sequences in the database. Indeed these proteins may indicate the primary advantage of our screening method, i.e. isolating atypical surface-related proteins, which would have been missed in all previously described screening protocols.

The construction of three reporter vectors and their use in *L. lactis* to identify and isolate genomic DNA fragments from pathogenic bacteria encoding secreted or surface associated proteins is now described.

Construction of the pTREP1-*nuc* series of reporter vectors

(a) <u>Construction of expression plasmid pTREP1</u>

The pTREP1 plasmid is a high-copy number (40-80 per cell) theta-replicating gram positive plasmid, which is a derivative of the pTREX plasmid which is itself a derivative of the the previously published pIL253 plasmid. pIL253 incorporates the

broad Gram-positive host range replicon of pAMβ1 (Simon and Chopin, 1988) and is non-mobilisable by the *L lactis* sex-factor. pIL253 also lacks the *tra* function which is necessary for transfer or efficient mobilisation by conjugative parent plasmids exemplified by pIL501. The Enterococcal pAMβ1 replicon has previously been transferred to various species including *Streptococcus, Lactobacillus* and *Bacillus* species as well as *Clostridium acetobutylicum*, (LeBlanc *et al.*, *Proceedings of the National Academy of Science USA* **75:**3484-3487 (1978)) indicating the potential broad host range utility. The pTREP1 plasmid represents a constitutive transcription vector.

The pTREX vector was constructed as follows. An artificial DNA fragment containing a putative RNA stabilising sequence, a translation initiation region (TIR), a multiple cloning site for insertion of the target genes and a transcription terminator was created by annealing 2 complementary oligonucleotides and extending with Tfl DNA polymerase. The sense and anti-sense oligonucleotides contained the recognition sites for NheI and BamHI at their 5' ends respectively to facilitate cloning. This fragment was cloned between the XbaI and BamHI sites in pUC19NT7, a derivative of pUC19 which contains the T7 expression cassette from pLET1 (Wells *et al., J. Appl. Bacteriol.* **74:**629-636 (1993)) cloned between the EcoRI and HindIII sites. The resulting construct was designated pUCLEX. The complete expression cassette of pUCLEX was then removed by cutting with HindIII and blunting followed by cutting with EcoRI before cloning into EcoRI and SacI (blunted) sites of pIL253 to generate the vector pTREX (Wells and Schofield, *In* Current advances in metabolism, genetics and applications-NATO ASI Series. **H 98:**37-62. (1996)). The putative RNA stabilising sequence and TIR are derived from the *Escherichia coli* T7 bacteriophage sequence and modified at one nucleotide position to enhance the complementarity of the Shine Dalgarno (SD) motif to the ribosomal 16s RNA of *Lactococcus lactis* (Schofield *et al.* pers. coms. University of Cambridge Dept. Pathology.).

A *Lactococcus lactis* MG1363 chromosomal DNA fragment exhibiting promoter activity which was subsequently designated P7 was cloned between the EcoRI and BglII sites present in the expression cassette, creating pTREX7. This active promoter region had been previously isolated using the promoter probe vector pSB292 (Waterfield *et al.*, *Gene* **165:**9-15 (1995)). The promoter fragment was amplified by PCR using the Vent DNA polymerase according to the manufacturer.

The pTREP1 vector was then constructed as follows. An artificial DNA fragment which included a transcription terminator, the forward pUC sequencing primer, a promoter multiple cloning site region and a universal translation stop sequence was created by annealing two overlapping partially complementary synthetic oligonucleotides together and extending with sequenase according to manufacturers instructions. The sense and anti-sense ($pTREP_F$ and $pTREP_R$) oligonucleotides contained the recognition sites for EcoRV and BamHI at their 5' ends respectively to facilitate cloning into pTREX7. The transcription terminator was that of the *Bacillus penicillinase* gene, which has been shown to be effective in Lactococcus (Jos *et al.*, *Applied and Environmental Microbiology* **50:**540-542 (1985)). This was considered necessary as expression of target genes in the pTREX vectors was observed to be leaky and is thought to be the result of cryptic promoter activity in the origin region (Schofield *et al.* pers. coms. University of Cambridge Dept. Pathology.). The forward pUC primer sequencing was included to enable direct sequencing of cloned DNA fragments. The translation stop sequence which encodes a stop codon in 3 different frames was included to prevent translational fusions between vector genes and cloned DNA fragments. The pTREX7 vector was first digested with EcoRI and blunted using the 5' - 3' polymerase activity of T4 DNA polymerase (NEB) according to manufacturer's instructions. The EcoRI digested and blunt ended pTREX7 vector was then digested with Bgl II thus removing the P7 promoter. The artificial DNA fragment derived from the annealed synthetic oligonucleotides was then digested with EcoRV and Bam HI and cloned into the EcoRI(blunted)-Bgl II digested pTREX7 vector to

generate pTREP. A *Lactococcus lactis* MG1363 chromosomal promoter designated P1 was then cloned between the EcoRI and BglII sites present in the pTREP expression cassette forming pTREP1. This promoter was also isolated using the promoter probe vector pSB292 and characterised by Waterfield *et al.*, (1995) [*supra*]. The P1 promoter fragment was originally amplified by PCR using vent DNA polymerase according to manufacturers instructions and cloned into the pTREX as an EcoRI-BglII DNA fragment. The EcoRI-BglII P1 promoter containing fragment was removed from pTREX1 by restriction enzyme digestion and used for cloning into pTREP (Schofield *et al.* pers. coms. University of Cambridge, Dept. Pathology.).

(b) PCR amplification of the *S. aureus nuc* gene.

The nucleotide sequence of the *S. aureus nuc* gene (EMBL database accession number V01281) was used to design synthetic oligonucleotide primers for PCR amplification. The primers were designed to amplify the mature form of the *nuc* gene designated *nuc*A which is generated by proteolytic cleavage of the N-terminal 19 to 21 amino acids of the secreted propeptide designated Snase B (Shortle, 1983 [*supra*]). Three sense primers (*nuc*S1, *nuc*S2 and *nuc*S3, shown in figure 3) were designed, each one having a blunt-ended restriction endonuclease cleavage site for EcoRV or SmaI in a different reading frame with respect to the *nuc* gene. Additionally BglII and BamHI were incorporated at the 5' ends of the sense and anti-sense primers respectively to facilitate cloning into BamHI and BglII cut pTREP1. The sequences of all the primers are given in figure 3. Three *nuc* gene DNA fragments encoding the mature form of the nuclease gene (*Nuc*A) were amplified by PCR using each of the sense primers combined with the anti-sense primer. The *nuc* gene fragments were amplified by PCR using *S. aureus* genomic DNA template, Vent DNA Polymerase (NEB) and the conditions recommended by the manufacturer. An initial denaturation step at 93°C for 2 min was followed by 30 cycles of denaturation at 93°C for 45 sec, annealing at 50°C for 45 seconds, and extension 73°C for 1 minute and then a final 5 min extension step

at 73°C. The PCR amplified products were purified using a Wizard clean up column (Promega) to remove unincorporated nucleotides and primers.

(c) <u>Construction of the pTREP1-*nuc* vectors</u>

The purified *nuc* gene fragments described in section b were digested with Bgl II and BamHI using standard conditions and ligated to BamHI and BglII cut and dephosphorylated pTREP1 to generate the pTREP1-*nuc*1, pTREP1-*nuc*2 and pTREP1-*nuc*3 series of reporter vectors. These vectors are described in figure 4. General molecular biology techniques were carried out using the reagents and buffers supplied by the manufacturer or using standard techniques (Sambrook and Maniatis, Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press: Cold Spring Harbour (1989)). In each of the pTREP1-*nuc* vectors the expression cassette comprises a transcription terminator, lactococcal promoter P1, unique cloning sites (BglII, EcoRV or SmaI) followed by the mature form of the *nuc* gene and a second transcription terminator. Note that the sequences required for translation and secretion of the *nuc* gene were deliberately excluded in this construction. Such elements can only be provided by appropriately digested foreign DNA fragments (representing the target bacterium) which can be cloned into the unique restriction sites present immediately upstream of the *nuc* gene.

(d) <u>Screening for secreted proteins in *Group B* Streptococcus.</u>

Genomic DNA isolated from and *Group B Streptococcus (S. agalactiae)* was digested with the restriction enzyme Tru9I. This enzyme which recognises the sequence 5'-TTAA -3' was used because it cuts A/T rich genomes efficiently and can generate random genomic DNA fragments within the preferred size range (usually averaging 0.5 - 1.0 kb). This size range was preferred because there is an increased probability that the P1 promoter can be utilised to transcribe a novel gene sequence. However, the P1 promoter may not be necessary in all cases as it is possible that many Streptococcal promoters are recognised in *L. lactis.* DNA fragments of different size ranges were

purified from partial Tru9I digests of and *S. agalactiae* genomic DNA. As the Tru 9I restriction enzyme generates staggered ends the DNA fragments had to be made blunt ended before ligation to the EcoRV or SmaI cut pTREP1-*nuc* vectors. This was achieved by the partial fill-in enzyme reaction using the 5'-3' polymerase activity of Klenow enzyme. Briefly Tru9I digested DNA was dissolved in a solution (usually between 10-20 μl in total) supplemented with T4 DNA ligase buffer (New England Biolabs; NEB) (1X) and 33 μM of each of the required dNTPs, in this case dATP and dTTP. Klenow enzyme was added (1 unit Klenow enzyme (NEB) per μg of DNA) and the reaction incubated at 25°C for 15 minutes. The reaction was stoped by incubating the mix at 75°C for 20 minutes. EcoRV or SmaI digested pTREP-*nuc* plasmid DNA was then added (usually between 200-400 ng). The mix was then supplemented with 400 units of T4 DNA ligase (NEB) and T4 DNA ligase buffer (1X) and incubated overnight at 16°C. The ligation mix was precipiated directly in 100% Ethanol and 1/10 volume of 3M sodium acetate (pH 5.2) and used to transform *L. lactis* MG1363 (Gasson, *J. Bacteriol.* **154:**1-9 (1983)). Alternatively, the gene cloning site of the pTREP-*nuc* vectors also contains a BglII site which can be used to clone for example Sau3AI digested genomic DNA fragments.

L. lactis transformant colonies were grown on brain heart infusion agar and nuclease secreting (*Nuc*$^+$) clones were detected by a toluidine blue-DNA-agar overlay (0.05 M Tris pH 9.0, 10 g of agar per litre, 10 g of NaCl per liter, 0.1 mM CaCl2, 0.03% wt/vol. salmon sperm DNA and 90 mg of Toluidine blue O dye) essentially as described by Shortle, 1983 [*supra*], and Le Loir *et al.*, 1994 [*supra*]). The plates were then incubated at 37°C for up to 2 hours. Nuclease secreting clones develop an easily identifiable pink halo. Plasmid DNA was isolated from *Nuc*$^+$ recombinant *L. lactis* clones and DNA inserts were sequenced on one strand using the *Nuc*Seq sequencing primer described in figure 3, which sequences directly through the DNA insert.

Whilst the example described above related specifically to *Group B Streptococcus*, it will be apparent to one skilled in the art that the same screening technique may be used to detect exported and secreted proteins in other gram positive bacteria, for example *Streptococcus pneumoniae*.

Example 2; Screening Group B Streptococcal derived genes in DNA vaccination experiments.

pcDNA3.1+ as a DNA vaccine vector

The commercially available pcDNA3.1+ plasmid (Invitrogen), referred to as pcDNA3.1 henceforth, was used as a vector in all DNA immunisation experiments involving gene targets derived using the LEEP system. pcDNA 3.1 is designed for high-level stable and transient expression in mammalian cells and has been used widely and successfully as a host vector to test candidate genes from a variety of pathogens in DNA vaccination experiments (Zhang *et al.*, 1997; Kurar and Splitter, 1997; Anderson *et al.*, 1996).

The vector possesses a multiple cloning site which facilitates the cloning of multiple gene targets downstream of the human cytomegalovirus (CMV) immediate-early promoter/enhancer which permits efficient, high-level expression of the target gene in a wide variety of mammalian cells and cell types including both muscle and immune cells. This is important for optimal immune response as it remains unknown as to which cells types are most important in generating a protective response *in vivo*. The plasmid also contains the ColE1 origin of replication which allows convenient high-copy number replication and growth in *E. coli* and the ampicillin resistance gene (B-lactamase) for selection in *E. coli.* In addition pcDNA 3.1 possesses a T7 promoter/priming site upstream of the MCS which allows for *in vitro* transcription of a cloned gene in the sense orientation.

Preparation of DNA vaccines

Oligonucleotide primers were designed for each individual gene of interest derived using the LEEP system. Each gene was examined thoroughly, and where possible, primers were designed such that they targeted that portion of the gene thought to

encode only the mature portion of the protein (**APPENDIX I**). It was hoped that expressing those sequences that encode only the mature portion of a target gene protein, would facilitate its correct folding when expressed in mammalian cells. For example, in the majority of cases primers were designed such that putative N-terminal signal peptide sequences would not be included in the final amplification product to be cloned into the pcDNA3.1 expression vector. The signal peptide directs the polypeptide precursor to the cell membrane via the protein export pathway where it is normally cleaved off by signal peptidase I (or signal peptidase II if a lipoprotein). Hence the signal peptide does not make up any part of the mature protein whether it be displayed on the bacterium's surface or secreted. Where a N-terminal leader peptide sequence was not immediately obvious, primers were designed to target the whole of the gene sequence for cloning and ultimately, expression in pcDNA3.1.

All forward and reverse oligonucleotide primers incorporated appropriate restriction enzyme sites to facilitate cloning into the pcDNA3.1 MCS region. All forward primers were also designed to include the conserved Kozak nucleotide sequence 5'-gccacc-3' immediately upstream of an 'atg' translation initiation codon in frame with the target gene insert. The Kozak sequence facilitates the recognition of initiator sequences by eukaryotic ribosomes. Typically, a forward primer incorporating a BamH1 restriction enzyme site the primer would begin with the sequence 5'-cgggatccgccaccatg-3', followed by a sequence homologous to the 5' end of that part of a gene being amplified. All reverse primers incorporated a Not I restriction enzyme site sequence 5' -ttgcggccgc-3'. All gene-specific forward and reverse primers were designed with compatible melting temperatures to facilitate their amplification.

All gene targets were amplified by PCR from *S. agalactiae* genomic DNA template using Vent DNA polymerase (NEB) or rT*th DNA* polymerase (*PE* Applied Biosystems) using conditions recommended by the manufacturer. A typical amplification reaction involved an initial denaturation step at 95°C for 2 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1 minute per kilobase of DNA being amplified). This was followed by a final extension period at 72°C for 10 minutes. All PCR amplified products were extracted once with phenol chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated.

Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). The purified amplification gene DNA fragments were digested with the appropriate restriction enzymes and cloned into the pcDNA3.1 plasmid vector using *E. coli* as a host. Successful cloning and maintenance of genes was confirmed by restriction mapping and by DNA sequencing. Recombinant plasmid DNA was isolated on a large scale (>1.5 mg) using Plasmid Mega Kits (Qiagen).

It was decided to include the *S. agalactiae rib* gene as a positive control in at least one trial of DNA immunisation experiments. Rabbit antiserum against the Rib protein (Stalhammar-Carleman *et al.*, 1993) and highly purified preparations of the Rib protein itself (Larsson *et al.*, 1999; Larsson *et al.*, 1996) have been shown to confer protection against lethal infection with strains expressing the antigen. All serotype III strains have been shown to express the Rib antigen and Southern blot analysis performed in the laboratory has confirmed that *S. agalactiae* serotype III (strain 97/0099) does contain the *rib* gene, hence the *rib* gene as part of a DNA vaccine would represent a potential positive control for all DNA immunisation experiments. Oligonucleotide primers were designed (**Appendix I**) that targeted only the mature portion of the *rib* gene and which included appropriate restriction enzyme sites for cloning into pcDNA3.1. *rib* was amplified using rT*th* DNA polymerase (*PE* Applied Biosystems) using conditions recommended by the manufacturer. Conditions for cloning were similar to that described previously.

Preparation of a *S. agalactiae* standard inoculum

Strain validation

S. agalactiae serotype III (strain 97/0099) is a recent clinical isolate derived from the cerebral spinal fluid of a new born baby suffering from meningitis. This haemolytic strain of Group B Streptococcus was epidemiologically tested and validated at the Respiratory and Systemic Infection Laboratory, PHLS Central Public health laboratory, 61 Collindale Avenue, London NW9 5HT. The strain was subcultured only twice prior to its arrival in the laboratory. Upon its arrival on a agar slope, a sweep of 4-5 colonies was immediately used to inoculate a Todd Hewitt/5% horse blood broth which was incubated overnight statically at 37 C. 0.5 ml aliquots of this overnight culture were then used to make 20% glycerol stocks of the bacterium for long term

storage at -70 C. Glyerol stocks were streaked on Todd Hewitt/5% horse blood agar plates to confirm viability.

In vivo passaging of Group B Streptoccocus

A frozen culture (described under strain validation) of *S. agalactiae* serotype III (strain 97/0099) was streaked to single colonies on Todd-Hewitt/5% blood agar plates which were incubated overnight at 37°C. A sweep of 4-5 colonies was used to inoculate a Todd Hewitt/5% horse blood broth which was again incubated overnight. A 0.5 ml aliquot from this overnight culture was used to inoculate a 50 ml Todd Hewitt broth (1:100 dilution) which was incubated at 37 C. 10-fold serial dilutions of the overnight culture were made (since virulence of this strain was unknown) and each were passaged intra-peritoneally (IP) in CBA/ca mice in duplicate. Viable counts were performed on the various inocula used in the passage. Groups of mice were challenged with various concentrations of the pathogen ranging from 10^8 to 10^4 colony forming units (cfu). Mice that developed symptoms were terminally anaesthetized and cardiac punctures were performed (Only mice that had been challenged with the highest doses, i.e. 1 X 10^8 cfu, developed symptoms). The retrieved unclotted blood was used to inoculate directly a 50ml serum broth (Todd Hewitt/20% inactivated foetal calf serum). The culture was constantly monitored and allowed to grow to late logarithmic phase. The presence of blood in the medium interfered with OD_{600} readings as it was being increasingly lysed with increasing growth of the bacterium, hence the requirement to constantly monitor the culture. Upon reaching late logarithmic phase/early stationary phase, the culture was transferred to a fresh 50 ml tube in order to exclude dead bacterial cells and remaining blood cells which would have sedimented at the bottom of the tube. 0.5 ml aliquots were then transferred to sterile cryovials, frozen in liquid nitrogen and stored at -70 C. A viable count was carried out on a single standard inoculum aliquot in order to determine bacterial numbers. This was determined to be approximately 5 X10^8 cfu per ml.

Intra-peritoneal Challenge and virulence testing of Group B Streptococcus standard inoculum

To determine if the standard inoculum was suitably virulent for use in a vaccine trial, challenges were carried out using a dose range. Frozen standard inoculum strain

aliquots were allowed to thaw at room temperature. From viable count data the number of cfu per ml was already known for the standard inoculum. Initially, serial dilutions of the standard inoculum were made in Todd Hewitt broth and mice were challenged intra-peritoneally with doses ranging from 1 X 10^8 to 1 X 10^4 cfu in a 500 µl volume of Todd Hewitt broth. The survival times of mouse groups injected with different doses of the bacterium were compared. The standard inoculum was determined to be suitably virulent and a dose of 1 X 10^6 cfu was considered close to optimal for further use in vaccine trials. Further optimisation was carried out by comparing mice challenged with doses ranging between 5 X 10^5 and 5 X 10^6 cfu. The optimal dose was estimated to be approximately 2.5 X10^6 cfu. This represented a 100% lethal dose and was repeatedly consistant with end-points as determined by survival times being clustered within a narrow time-range. Throughout all these experiments, challenged mice were constantly monitored to clarify symptoms, stages of symptom development as well as calculating survival times.

Vaccine trials

Vaccine trials in mice were accomplished by the administration of DNA to 6 week old CBA/ca mice (Harlan, UK). Mice to be vaccinated were divided into groups of six and each group were immunised with recombinant pcDNA3.1 plasmid DNA containing a specific target-gene sequence derived using the LEEP system. A total of 100 µg of DNA in Dulbecco's PBS (Sigma) was injected intramuscularly into the tibialis anterior muscle of both hind legs. Four weeks later this procedure was repeated using the same amount of DNA. For comparison, control mice groups were included in all vaccine trials. These control groups were either not DNA-vaccinated or were immunised with non-recombinant pcDNA3.1 plasmid DNA only, using the same time course described above. Four weeks after the second immunisation, all mice groups were challenged intra-peritoneally with a lethal dose of *S. agalactiae* serotype III (strain 97/0099). The actual number of bacteria administered was determined by plating serial dilutions of the inoculum on Todd-Hewitt/5% blood agar plates. All mice were killed 3 or 4 days after infection. During the infection process, challenged mice were monitored for the development of symptoms associated with the onset of *S. agalactiae* induced-disease. Typical symptoms in an appropriate order included piloerection, an increasingly hunched posture, discharge from eyes, increased lethargy and reluctance to move which was often the result of apparent paralysis in the lower body/hind leg region. The

latter symptoms usually coincided with the development of a moribund state at which stage the mice were culled to prevent further suffering. These mice were deemed to be very close to death, and the time of culling was used to determine a survival time for statistical analysis. Where mice were found dead, a survival time was calculated by averaging the time when a particular mouse was last observed alive and the time when found dead, in order to determine a more accurate time of death.

Interpretation of Results

A positive result was taken as any DNA sequence that was cloned and used in challenge experiments as described above and gave protection against that challenge. DNA sequences were determined to be protective;

-if that DNA sequence gave statistically significant protection (to a 95% confidence level ($p>0.05$) as determined using the Mann-Whitney U test.

-if that DNA sequence was marginal or non-signficant using Mann-Whitney but showed some protective features. For example, one or more outlying mice may survive for significantly longer time periods when compared with control mice. Alternatively, the time to first death may also be prolonged when compared to counterpart mice in control groups.

It is acceptable to allow marginal or non-significant results to be considered as potential positives when it is possible that the clarity of some results may be affected by problems associated with the administration of the DNA vaccine. Indeed, much varied survival times may reflect different levels of immune response between different members of a given group.

Results

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 1 (Figure 4a)

	Mean Survival Times (hours)				
	pcDNA3.1	17(ID-8)	18(ID-9)	20(ID-25)	rib
1	26.833	14.916	27.750	30.500	88.666

2	42.333	94.000 **(T)**	34.333	33.333	28.166
3	47.916	45.166	41.083	34.083	37.250
4	28.333	30.750	47.083	23.500	37.250
5	42.333	74.666	94.000 **(T)**	94.000 **(T)**	94.000 **(T)**
6	25.333	25.000	26.166	30.500	45.750
Mean	**37.549**	**51.899**	**48.849**	**43.083**	**57.066**
sd	**9.3943**	**32.214**	**26.257**	**28.768**	**31.556**
p value 1		**0.4049**	**0.4049**	**0.5000**	**0.1481**
p value 2	**> 39.0**	**> 39.0**	**> 39.0**	**> 39.0**	

(T) - terminated at conclusion of experiment but showing symptoms of infection.

p value 1 refers to statistical significance when compared to pcDNA3.1 controls.

p value 2 refers to statistical significance when compared to rib positive control.

All DNA vaccine's showed a pattern of protection similar to that obtained with the rib DNA vaccine, which was initially used as a positive control.

17 (ID-8)

Mice immunised with the '17 (ID-8)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there are two outlying mice one of which survived the term of the experiment despite developing symptoms. The group also exhibited a much wider range of survival times reflected by a mean survival value which is approximately 14 hours higher than that demonstrated by the unvaccinated control group.

18 (ID-9)

Mice immunised with the '18 (ID-9)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there is one outlying mouse which survived the term of the experiment despite developing symptoms.

20 (ID-25)

Mice immunised with the '20 (ID-25)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there was one outlying mouse which survived the term of the experiment despite developing symptoms.

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 2 (Figure 4b)

	Mean Survival Times (hours)			
	pcDNA	UnVacc	22(ID-10)	28(ID-13)
1	45.000	27.916	44.666	72.000 **(T)**
2	37.333	45.083	51.416	33.000
3	37.333	37.583	40.791	36.083
4	35.291	24.583	44.666	72.000 **(T)**
5	24.333	37.583	36.916	49.166
6	45.000	33.166	57.833	36.083
Mean	**35.858**	**34.549**	**43.691**	**52.449**
sd	**7.4342**	**8.2567**	**5.3825**	**18.850**
p value 1		**> 39.0**	**0.1137**	**0.2340**
p value 2	**0.4679**		**0.0323**	**0.1137**

(T) - terminated at conclusion of experiment but showing symptoms of infection.

p value 1 refers to statistical significance when compared to pcDNA3.1 controls.

p value 2 refers to statistical significance when compared to unvaccinated controls.

There was no significant difference in the survival times exhibited by the pcDNA3.1 and unvaccinated control groups. This is confirmed by their very similar mean survival times of 35.858 hours (pcDNA3.1) and 34.166 hours (Unvaccinated).

22 (ID-10)

Mice immunised with the '22 (ID-10)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group but not when compared with the pcDNA3.1 control group. In addition, the time to first death in this group was prolonged by approximately 12 hours when compared to the pcDNA3.1 and unvaccinated control groups. The mean survival time of 43.691 hours is also considerbly higher than that determined for both control groups.

28 (ID-13)

Mice immunised with the '28 (ID-13)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 and unvaccinated control groups. However there are three outlying mice, two of which survived the term of the experiment despite showing symptoms. In addition, the time to first death in this group was prolonged by approximately 9 hours when compared to the pcDNA3.1 and unvaccinated control groups. The mean survival time of 52.449 hours is also considerbly higher than that determined for both control groups, as well demonstrating a wider range of survival times.

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 3 (Figure 4c)

	Mean Survival Times (hours)				
	UnVacc.	70(ID-42)	94(ID-48)	86(ID-47)	51(ID-37)
1	27.583	25.166	34.666	32.416	43.749
2	27.583	42.666	49.500	32.416	38.333
3	24.583	34.666	27.000	42.500	50.666
4	22.250	42.666	30.500	34.500	45.166
5	35.916	30.583	30.500	34.500	69.082
6	22.250	25.166	42.666	42.500	31.166
Mean	**27.583**	**35.149**	**34.433**	**35.266**	**49.399**
sd	**5.1691**	**7.6444**	**8.8495**	**4.1758**	**11.846**
p value		**0.0628**	**0.0321**	**0.0153**	**0.0041**

p value refers to statistical significance when compared to unvaccinated controls.

70 (ID-42)

Mice immunised with the '70 (ID-42)' DNA vaccine, marginally did not show significantly longer survival times when compared with the unvaccinated control group. However, the first death in this group is prolonged (by approximately 3 hours) when compared with the unvaccinated group. In addition, the group has a mean survival time which is approximately 8 hours longer than the unvaccinated group.

94 (ID-48)

Mice immunised with the '94 (ID-48)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

86 (ID-47)

Mice immunised with the '86 (ID-47)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

51 (ID-37)

Mice immunised with the '51 (ID-37)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 4 (Figure 4d)

	Mean Survival Times (hours)	
	UnVacc	9(ID-6)
1	32.666	35.250
2	21.666	30.958
3	23.916	69.333
4	22.999	52.333
5	25.916	44.916
6	35.916	47.083
Mean	**25.432**	**46.041**
sd	**4.3291**	**16.096**
p value		**0.0101**

(T) - terminated at conclusion of experiment but showing symptoms of infection.

p value refers to statistical significance when compared to unvaccinated controls

9 (ID-6)

Mice immunised with the '39(ID-6)' DNA vaccine showed significantly longer survival times when compared with the control group.

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 6 (Figure 4e)

	Mean Survival Times (hours)				
	pcDNA	UnVacc	32 (ID-15)	39(ID-17)	57(40)
1	33.541	36.000	25.041	52.333	28.333
2	36.750	29.999	30.458	44.750	32.708
3	36.750	32.749	44.833	44.750	36.083
4	36.750	44.500	30.458	36.250	40.333
5	29.000	28.333	64.833	36.250	72.000 **(T)**
6	30.750	31.666	72.000 **(T)**	28.583	33.750
Mean	**34.558**	**34.316**	**39.124**	**44.016**	**38.103**
sd	**3.4036**	**6.3921**	**16.140**	**13.833**	**12.986**
p value 1		**> 39.0**	**0.4043**	**0.1867**	**0.4044**
p value 2	**0.2862**		**0.2873**	**0.0458**	**0.2113**

(T) - terminated at conclusion of experiment but showing symptoms of infection.

p value 1 refers to statistical significance when compared to pcDNA3.1 controls

p value 2 refers to statistical significance when compared to unvaccinated controls.

There was no significant difference in the survival times exhibited by the pcDNA3.1 and unvaccinated control groups. This is confirmed by their

very similar mean survival times of 34.558 hours (pcDNA3.1) and 34.316 hours (Unvaccinated).

32 (ID-15)

Mice immunised with the '32 (ID-15)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 and unvaccinated control groups. However, the '32 (ID-15)' group has two outlying mice one of which survived the term of the experiment despite showing symptoms. This group also exhibits a wide range of survival times.

39 (ID-17)

Mice immunised with the '39 (ID-17)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group but was not significant when compared with the pcDNA3.1 control group. The group has a considerbly higher mean survival time of 44.016 hours than that determined for either of the control groups.

57 (ID-40)

Mice immunised with the '32 (ID-15)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 and unvaccinated control groups. However, the '32 (ID-15)' group has one outlying mouse which survived the term of the experiment despite showing symptoms.

SURVIVAL DATA-SET B

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 2 (Figure 5a)

	Mean Survival Times (hours)		
	pcDNA	UnVacc	13(ID-72)
1	45.000	27.916	69.166
2	37.333	45.083	36.333
3	37.333	37.583	43.916
4	35.291	24.583	32.166
5	24.333	37.583	36.333
6	45.000	33.166	43.916
Mean	35.858	34.549	43.582
sd	7.4342	8.2567	14.917
p value 1		> 39.0	0.4679
p value 2	0.4679		0.1880

p value 1 refers to statistical significance when compared to pcDNA3.1 controls.

p value 2 refers to statistical significance when compared to unvaccinated controls.

There was no significant difference in the survival times exhibited by the pcDNA3.1 and unvaccinated control groups. This is confirmed by their very similar mean survival times of 35.858 hours (pcDNA3.1) and 34.166 hours (Unvaccinated).

13 (ID-72)

Mice immunised with the '13 (ID-72)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 and unvaccinated control groups. However, there is one outlying mouse which survived approximately 24 hours longer than the longest surviving mice in the pcDNA3.1 and unvaccinated control groups respectively. In addition, the time to first death in this group was prolonged when

compared to the pcDNA3.1 and unvaccinated control groups. The mean survival time of 43.582 hours is considerbly higher than that determined for both control groups.

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 3 (Figure 5b)

	Mean Survival Times (hours)		
	UnVacc	3-60(ID-65)	3-5(ID-66)
1	27.583	54.416	42.916
2	27.583	31.000	42.916
3	24.583	43.000	32.874
4	22.250	34.916	42.916
5	35.916	38.958	27.333
6	22.250	34.916	30.916
Mean	**27.583**	**40.458**	**37.791**
sd	**5.1691**	**8.9959**	**7.2860**
p value		**0.0098**	**0.0215**

p value refers to statistical significance when compared to unvaccinated controls.

3-60 (ID-65)

Mice immunised with the '3-60 (ID-65)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

3-5 (ID-66)

Mice immunised with the '3-5 (ID-66)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 4 (Figure 5c)

	Mean Survival Times (hours)			
	UnVacc	3-40(ID-67)	3-30(ID-68)	3-38(ID-69)
1	32.666	79.750	35.500	68.583
2	21.666	35.833	28.333	29.916
3	23.916	30.500	31.208	29.916
4	22.999	22.708	98.000 **(T)**	31.041
5	25.916	28.583	73.500	32.166
6	35.916	40.791	32.333	29.916
Mean	**25.432**	**39.474**	**53.308**	**38.324**
sd	**4.3291**	**22.998**	**30.961**	**16.940**
p value		**0.1149**	**0.0463**	**0.1132**

(T) - terminated at conclusion of experiment but showing symptoms of infection.

p value refers to statistical significance when compared to unvaccinated controls

3-40 (ID-67)

Mice immunised with the '3-40 (ID-67)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there is one outlying mouse which survived approximately 43 hours longer than the longest surviving mice in the unvaccinated control group.

3-30 (ID-68)

Mice immunised with the '3-30 (ID-68)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

3-38 (ID-69)

Mice immunised with the '2-19 (ID-73)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there was one outlying mouse which survived approximately 32 hours longer than the longest surviving mice in the unvaccinated control group. In addition, the time to first death was prolonged (by approximately 8 hours) when compared to the unvaccinated controls.

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 5 (Figure 5d)

	Mean Survival Times (hours)				
	UnVacc	141(ID-70)	3-20(ID-71)	2-19(ID-73)	3-6(ID-74)
1	27.833	47.500	36.166	36.166	44.666
2	45.666	52.833	44.833	49.833	36.000
3	45.666	49.333	26.750	55.833	75.416
4	34.333	46.250	36.166	68.583	36.000
5	34.333	47.500	55.916	33.333	55.916
6	45.666	36.500	44.833	30.583	36.000
Mean	**37.566**	**48.683**	**37.234**	**48.749**	**49.599**
sd	**7.8558**	**2.5672**	**8.4103**	**14.497**	**16.587**
p value		**0.0101**	**0.5000**	**0.2336**	**0.1854**

p value - refers to statistical significance when compared to unvaccinated controls.

141 (ID-70)

Mice immunised with the '141 (ID-70)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

3-20 (ID-71)

Mice immunised with the '3-20 (ID-71)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there is one outlying mouse which survived approximately 10 hours longer than the longest surviving mice in the unvaccinated control group.

2-19 (ID-73)

Mice immunised with the '2-19 (ID-73)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there are three outlying mouse which survived approximately 4, 10 and 23 hours longer than the longest surviving mice in the unvaccinated control group. This is reflected in the higher mean survival time of 48.749 hours and a much wider range of survival times.

3-6 (ID-74)

Mice immunised with the '3-6 (ID-74)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there are three outlying mouse which survived approximately 4, 10 and 23 hours longer than the longest surviving mice in the unvaccinated control group. This is reflected in the higher mean survival time of 49.599 hours and a much wider range of survival times.

Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 6 (Figure 5e)

	Mean Survival Times (hours)			
	pcDNA3.1	UnVacc.	3-51(ID-75)	3-56 (ID-76)

1	33.541	36.000	36.333	46.583
2	36.750	29.999	30.291	29.833
3	36.750	32.749	32.000	40.166
4	36.750	44.500	52.333	46.583
5	29.000	28.333	72.000 (T)	46.583
6	30.750	31.666	40.499	---
Mean	**34.558**	**34.316**	**44.591**	**40.791**
sd	**3.4036**	**6.3921**	**16.615**	**7.9070**
p value 1		**> 39.0**	**0.1876**	**0.0386**
p value 2	**0.2862**		**0.0867**	**0.0587**

(T) - terminated at conclusion of experiment but showing symptoms of infection.

p value 1 refers to statistical significance when compared to pcDNA3.1 controls

p value 2 refers to statistical significance when compared to unvaccinated controls.

There was no significant difference in the survival times exhibited by the pcDNA3.1 and unvaccinated control groups. This is confirmed by their very similar mean survival times of 34.558 hours (pcDNA3.1) and 34.316 hours (Unvaccinated).

3-51 (ID-75)

Mice immunised with the '3-51 (ID-75)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 control group but was relatively close to significant when compared with the unvaccinated control group. The '3-51' group has two outlying mouse one of which survived the term of the experiment despite developing symptoms. The mean survival time of 44.499 hours is considerbly higher than that determined for both control groups and the group also demonstrates as a much wider range of survival times.

3-56 (ID-76)

Mice immunised with the '3-56 (ID-76)' DNA vaccine exhibited significantly longer survival times when compared with the pcDNA3.1 control group but were marginally not significant when compared with unvaccinated control group.

Example 3: Conservation and varability of candidate vaccine antigen genes among different isolates of Group B Strepococci

An initial Southern blot analysis was carried out to determine cross-serotype conservation of novel Group B Streptococcal genes isolated using the LEEP system. Analysing the serotype distribution of a target gene will also determine their potential use as antigen components in a GBS vaccine. The Group B Streptococcal strains whose DNA was analysed as part of this study are listed in **APPENDIX II**.

Amplification and labelling of specific target genes as DNA probes for Southern blot analysis.
Oligonucleotide primers were designed for each individual gene of interest derived using the LEEP system. Primers were designed to target the whole of the gene being investigated (All primers are listed in **APPENDIX III**). Specific gene targets were amplified by PCR using Vent DNA polymerase (NEB) according to the manufacturers instructions. Typical reactions were carried out in a 100 µl volume containing 50 ng of GBS template DNA, a one tenth volume of enzyme reaction buffer, 1 µM of each primer, 250 µM of each dNTP and 2 units of Vent DNA polymerase. A typical reaction contained an initial 2 minute denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1 minute per kilobase of DNA being amplified). The annealing temperature was determined by the lower melting temperature of the two oligonucleotide primers. The reaction was concluded with a final extension period of 10 minutes at 72°C.

All PCR amplified products were extracted once with phenol chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated. Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). For use as DNA probes, purified amplified gene DNA fragments were labelled with digoxygenin using the DIG Nucleic Acid Labelling Kit (Boehringer Mannheim) according to the manufacturer's instructions.

Southern blot hybridisation analysis of Group B Streptococcal genomic DNA

Genomic DNA had previously been isolated from all strains of Group B Streptococci which were investigated for conservation of LEEP-derived gene targets. Appropriate DNA concentrations were digested using either *Hin* DIII, *Eco* RI or *Bgl* IIrestriction enzymes (NEB) according to manufacturer instructions and analysed by agarose gel electrophoresis. Following agarose gel electrophoresis of DNA samples, the gel was denatured in 0.25M HCl for 20 minutes and DNA was transferred onto Hybond™ N^+ membrane (Amersham) by overnight capillary blotting. The method is essentially as described in Sambrook *et al.* (1989) using Whatman 3MM wicks on a platform over a reservoir of 0.4M NaOH. After transfer, the filter was washed briefly in 2x SSC and stored at 4 C in Saran wrap (Dow chemical company).

Filters were prehybridised, hybridised with the digoxygenin labelled DNA probes and washed using conditions recommended by Boehringer Mannheim when using their DIG Nucleic Acid Detection Kit. Filters were prehybridised at 68°C for one hour in hybridisation buffer (1% w/v supplied blocking reagent, 5x SSC, 0.1% v/v N-lauryl sarcosine, 0.02% v/v sodium dodecyl sulphate[SDS]). The digoxygenin labelled DNA probe was denatured at 99.9°C for 10 minutes before being added to the hybridisation buffer. Hybridisation was allowed to proceed overnight in a rotating Hybaid tube in a Hybaid Mini-hybridisation oven. Unbound probe was removed by washing the filter twice with 2x SSC- 0.1% SDS for 5 minutes at room temperature. For increased stringency filters were then washed twice with 0.1x SSC-0.1% SDS for 15 minutes at 68°C. The DIG Nucleic Acid Detection Kit (Boehringer Mannheim) was used to immunologically detect specifically bound digoxygenin labelled DNA probes.

Results of Southern blot analysis

All genomic digests and their corresponding Southern blots followed an identical lane order as described in Table I.

Table I

Lane	Strain	Serotype
1	1 kb molecular Weight Marker	
2	515	Ia
3	A909	Ia
4	SB35	Ib
5	H36B	Ib
6	18RS21	II
7	1954/92	II
8	118/158	II
9	97/0057	II
10	BM110	III
11	BS30	III
12	M781	III
13	97/0099	III
14	3139	IV
15	1169-NT	V
16	GBS 6	VI
17	7271	VII
18	JM9	VIII
19	Group A Strepococcus	_
20	*Streptococcus pneumoniae*	14

For comparative purposes, it was decided to analyse the serotype distribution of the GBS *rib* gene, which encodes the known protective immunogen Rib. Rib has previously been shown to be present in serotype III and some strains of serotype II but not in serotypes Ia or Ib (Stalhammar-Carlemalm *et al.*, 1993). Confirmation of this pattern would not only give increased confidence in interpreting subsequent results, it would also determine if a *rib* gene homologue was present in the remaining GBS

serotypes being investigated here. Primers designed for the amplification of *rib* and its subsequent cloning into pcDNA3.1 (**Appendix I**), were used to generate a *rib* gene probe for Southern blot analysis.

Southern blot analysis - *rib* (Figure 6)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Genomic DNA from each strain was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled *rib* gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

<u>Comment</u>

The Southern blot analysis described in Figure 7 indicates that the *rib* gene is not conserved across all GBS serotypes. *rib* appears to be absent from all serotype Ia and Ib strains (lanes 2 to 5) and from strains 118/158 and 97/0057 of serotype II (lanes 8 and 9). However, *rib* would appear to present in strains 18RS21 and 1954/92 of serotype II (lanes 6 and 7) and in all strains of serotype III (lanes 10 to 13). This is in agreement with previously published data (Stalhammar-Carlemalm *et al.*, 1993). *rib* would also appear to be present in strains representing serotypes VII and VII (lanes 17 and 18) but was absent from strains representing serotypes IV, V and V (lanes 14 to 16) as well as the control strains (lanes 19 and 20). The *rib* gene probe did hybridise with lower intensity to genomic DNA fragments from strains representing serotypes Ia, Ib, IV, VI, VII and serotype II strains 118/158 and 97/0057. This may indicate the presence of a gene in these strains with a lower level of homology to *rib*. These hybridising DNA fragments may contain a homologue of the GBS *bca* gene encoding the Ca protein antigen which has been shown to be closely homologous to the Rib protein (Wastfelt *et al.*, 1996). If this is the case, it would be in agreement with previous work which showed all strains of serotypes Ia, Ib, II and III to be positive for one the two proteins (Stalhammar-Carlemalm *et al.*, 1993). However, the apparent

variable distribution of the *rib* gene amongst different GBS serotypes, makes it a less than ideal candidate for use in a GBS vaccine that is cross-protective against all serotypes.

Southern blot analysis - 4 (ID-1) (photograph 7)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Genomic DNA from each strain was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled 4 (ID-1) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

<u>Comment</u>

The Southern blot analysis described in Figure 7 indicates that gene 4 (ID-1) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Hin* DIII-digested genomic DNA fragment of approximately 3.5 kb in DNA digests from all GBS representatives. but was absent from both the control strains (lanes 19 and 20).

Southern blot analysis - 5 (ID-2) (Figure 8)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Genomic DNA from each strain was digested completely with *Eco* RI (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled 5 (ID-2) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

<u>Comment</u>

The Southern blot analysis described in Figure 7 indicates that gene 4 (ID-1) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Eco* RI-digested genomic DNA fragment of approximately 6.2 kb in DNA digests from all GBS representatives. but was absent from both the control strains (lanes 19 and 20).

Southern blot analysis - 15 (ID-7) (Figure 9)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Genomic DNA from each strain was digested completely with *Eco* RI (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled 15 (ID-7) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

<u>Comment</u>

The Southern blot analysis described in Figure 7 indicates that gene 15 (ID-7) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Eco* RI-digested genomic DNA fragment of approximately 6.2 kb in DNA digests from all GBS representatives. but was absent from both the control strains (lanes 19 and 20). The gene probe hybridised specifically with *Eco* RI -digested DNA fragments ranging from approximately 3.5 kb to 5.2 kb in size.

Southern blot analysis - 17 (ID-8) (Figure 10)

Figure 5

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Genomic DNA from each strain was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled

17 (ID-8) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Comment

The Southern blot analysis described in Figure 7 indicates that gene 17 (ID-8) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Hin* DIII-digested genomic DNA fragment of approximately 2.3 kb in DNA digests from all GBS representatives. but was absent from both the control strains (lanes 19 and 20).

Southern blot analysis - 22 (ID-10) (Figure 11)

Figure 6

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Genomic DNA from each strain was digested completely with *Bgl* II (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N^+ (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled 22 (ID-10) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Comment

The Southern blot analysis described in Figure 7 indicates that gene 22 (ID-10) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Bgl* II-digested genomic DNA fragment of approximately 3.1 kb in DNA digests from all GBS representatives except serotype Ib strain H36B, where the gene probe hybridised specifically to a a *Bgl* II-digested genomic DNA fragment. Gene 22 (ID-10) was absent from both the control strains (lanes 19 and 20).

Conclusion

The Southern blot analyses described here, represents a preliminary investigation into the conservation level of LEEP-derived genes amongst different GBS serotypes. Initial results indicate that the genes 4 (ID-1), 5 (ID-2), 15 (ID-7), 17(ID-8) and 22

(ID-10) are present in all GBS serotypes and thus represent potential candidate genes for use in a GBS vaccine. Similar analyses are being currently carried out for each of the genes contained in this patent.

APPENDIX I

ID-8 (17)
Forward Primer
5' - cgggatccgccaccatgACCACTTCTCAAGCTGTTTTAGC - 3'
Reverse Primer
5' - ttgcggccgcACGATTATCAACAAAGTTCTG - 3'

ID-9 (18)
Forward Primer
5' - cggatccgccaccatgGCTACTCATATTGGAAGTTACCAGC - 3'
Reverse Primer
5' -ttgcggccgcAGGGTTTATTTGTTGAAGTGTCTTG - 3'

ID-10 (22)
Forward Primer
5' - cggatccgccaccatgTATCTATATCATTTACCAATGCCC -3'
Reverse Primer
5' - ttgcggccgcTTTATGTATAGAAACAGCAGTCCC -3'

ID-13 (28)
Forward Primer
5' - cggatccgccaccatgAAAGGAAGAACAACCTATTCGTTTAG -3'
Reverse Primer
5' - ttgcggccgcAAGAGCAAATTTTCGTATCTCCTC -3'

ID-15 (32)
Forward Primer
5' - cggatccgccaccATGATTGTTGGACACGGAATTG -3'
Reverse Primer
5' - ttgcggccgcTTTTTCTTCCTCCAAAATAACACTAGC -3'

ID-17 (39)
Forward Primer

5' - cggatccgccaccatgGCGACTAAAGAGTTAGGTGTTAG -3'

Reverse Primer

5' - ttgcggccgcTATAGTTTTAGTTTCAACTTGTCTAGATG -3'

ID-25 (20)

Forward Primer

5' - cgggatccaccatgTATACGAGTTTACAACCAAATCATG -3'

Reverse Primer

5' - ttgcggccgcGTCAGCTCGTACTGTTTTTTTAGC -3'

ID-37 (51)

Forward Primer

5' - cggatccgccaccatgTGTCAAATGAATAGTGAACATAAAAG -3'

Reverse Primer

5' - ttgcggccgcCTCAAATAATTTACCTCCAATTCG -3'

ID-40 (51)

Forward Primer

5' - cggatccgccaccatgGCTCCATTCGAATTTAAAGATTC -3'

Reverse Primer

5' - ttgcggccgcTGATTTACCAGTTTGGAAGAGTTC -3'

ID-42 (70)

Forward Primer

5' - cggatccgccaccATGAATACTATTTATAATACATTGAGAACAG -3'

Reverse Primer

5' - ttgcggccgcTTCTTTGTTCCAACTTTCTGG -3'

ID-47 (86)

Forward Primer

5' - cggatccgccaccATGATAGAGTGGATTCAAACACATTTAC -3'

Reverse Primer

5' - ttgcggccgcTTTATGACTCAAGCGACGTGTTA -3'

ID-48 94

Forward Primer

5' - cggatccgccaccATGGAGTTAGTAATTAGAGATATTCGTAAG

Reverse Primer

5' - ttgcggccgcCTTGTCATATTCATCTCCCTTCAAC

ID-67 (3-40)

Forward Primer

5' - cggatccgccaccatgGCTAGTTTTGTCATGAATCATAATGAC -3'

Reverse Primer

5' - ttgcggccgcGTTATTTGCTCGTTGTTTAGCTAAATC -3'

ID-68 (3-30)

Forward Primer

5' - cggatccgccaccatgGCTCTTAGTTTTTTATGGTTTCAGTTCAAGC -3'

Reverse Primer

5' - ttgcggccgcGAAGGCACCGCCACCTCC -3'

ID-69 (3-38)

Forward Primer

5' - cggatccgccaccatgGGTGAAACCCAAGATACCAATCAAGC -3'

Reverse Primer

5' - ttgcggccgcAACACCTGGTGGGCGTTTGG -3'

ID-70 (141)

Forward Primer

5' - cggatccgccaccATGGCTGGGAATCGTAATAACG -3'

Reverse Primer

5' - ttgcggccgcAGCCGTCTCTAAAACAGGCTTG -3'

ID-71 (3-20)

Forward Primer

5' - cggatccgccaccatgCTTCCAACGCAGCCGCAAAAC -3'

Reverse Primer

5' - ttgcggccgcATTTAGTGTTATTTCTCCTGTTGCATAATCC -3'

ID-72 (13)

Forward Primer

5' - cgggatccaccatgTACACGCATATTGTTGAAAAAAG -3'

Reverse Primer

5' - ttgcggccgcAAATAATTTCTTTTGGTTGTTTG -3'

ID-73 (2-19)

Forward Primer

5' - cggatccgccaccatgAGTAATCAAGAAGTTTCAGCAAGC -3'

Reverse Primer

5' - ttgcggccgcCCATTGTGGAATATCAGCTGAAG -3'

ID-74 (3-6)

Forward Primer

5' - cggatccgccaccatgGTGCAGGCAGTGGTACCGCT -3'

Reverse Primer

5' - ttgcggccgcGCGCATTGTAACAAATTCCTCAG -3'

ID-75 (3-51)

Forward Primer

5' - cgggatccaccatgGCTGCCGAGAAGGATAAAG -3'

Reverse Primer

5' - ttgcggccgcATTATTTAGCTGCTTTTTTAATGG -3'

ID-76 (3-56)

Forward Primer

5' - cgggatccaccatgTGTCAGGTTGTTTATGCAAGTTTTC -3'

Reverse Primer

5' - ttgcggccgcTTTACTAATTGATAAAGAGCAACTTCG -3'

rib (control)

Forward primer
5' - ggggtaccggccaccATGGCTGAAGTAATTTCAGGAAGT -3'
Reverse primer
5' - cggaattccgTTAATCCTCTTTTTTTCTTAGAAACAGAT

APPENDIX II

Listed below are the details (serotype and strain designation) of Group B Streptococcus strains whose DNA was analysed for gene conservation

SEROTYPE	STRAIN
Ia	515
Ia	A909
Ib	SB35
Ib	H36B
II	18RS21
II	1954/92
II	118/158
II	97/0057
III	BM110
III	BS30
III	M781
III	97/0099
IV	3139
V	1169/NT
VI	GBS VI
VII	7271
VIII	JM9

A group A Streptococcal strain (serotype M1, strain NCTC8198) and *Streptococcus pneumoniae* (serotype 14) were also included in the analysis for control purposes.

APPENDIX III

ID-1 (4)
forward primer
5' - atggaaaaaaatacttggaaaaaattac -3'
reverse primer
5' - ctattttgttttagcgatgtctttatc -3'

ID-2 (5)
forward primer
5' - atgtcaaaacaaaaagtaacggcaac -3'
reverse primer
5' - ttatttatggccaataccataagttaattg

ID-6 (9)
forward primer
5' - atgaaaaaagtttttttctcatggctatg -3'
reverse primer
5' - ttacttcaactgttgatagagcacttcc - 3'

ID-7 (15)
forward primer
5' - ttgttcaattttataggttttagaacttgg -3'
reverse primer
5' - ttaattttcattgcgtctcaaacc -3'

ID-8 (17)
forward primer
5' - atgacaaaaaaacttattattgctatattag -3'
reverse primer
5' - ttaacgattatcaacaaagttctgtac -3'

ID-10 (22)
forward primer

5' - atgatacgccagtttttaagagaa -3'
reverse primer
5' - ttatttatgtatagaaacagcagtccc -3'

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Larsson, C., Stalhammar-Carlemalm, M., and Lindahl, G. 1999. Protection against experimental infection with Group B Streptococcus by immunization with a bivalent protein vaccine. *Vaccine.* **17**:454-458

Stalhammar-Carlemalm, M., Stenberg, L., and Lindahl, G. 1993. Protein Rib: a novel Group B Streptococcal protein that confers protective immunity and is expressed by most strains causing invasive infections: *J. Exp. Med.* **177**: 1593-1603

Wastfelt, M., Stalhammar-Carlemalm, M., (1996) Identification of a family of Streptococcal surface proteins with extremely repetitive structure. *J. Biol. Chem.* **271**: 18892-18897.

Zhang, D., Yang, X., Berry, J. Shen, C., McClarty, G. and Brunham, R.C. (1997) DNA vaccination with the major outer-membrane protein genes induces acquired immunity to *Chlamydia trachomatis* (mouse pneumonitis) infection. *Infection and Immunity*, **176**, 1035-40.

Claims:

1. A *Group B Streptococcus* protein having a sequence selected from those described in fig 1, or fragments or derivatives thereof.

2. A *Group B Streptococcus* polypeptide or peptide having a sequence selected from those described in fig 2, or fragments or derivatives thereof.

3. Derivatives or variants of the proteins, polypeptides, and peptides as claimed in claims 1 and 2 which show at least 50% identity to those proteins, polypeptides and peptides claimed in claims 1 and 2.

4. A nucleic molecule comprising or consisting of a sequence which is:

(i) any of the DNA sequences set out in figure 1 and figure 2 herein or their RNA equivalents;

(ii) a sequence which is complementary to any of the sequences of (i);

(iii) a sequence which codes for the same protein or polypeptide, as those sequences of (i) or (ii);

(iv) a sequence which shows substantial identity with any of those of (i), (ii) and (iii); or

(v) a sequence which codes for a derivative, or fragment of a nucleic acid molecule shown in figure 1 or figure 2.

5. A vector comprising DNA encoding for the expression of any one or more proteins, polypeptides, peptides, fragments or derivitives thereof, as claimed in claims 1 to 3.

6. A vector as claimed in claim 5 further comprising DNA encoding any one or more of the following: promoters, enhancers, signal sequences, leader sequences,

translation start and stop signals, DNA stability controlling regions, or a fusion partner.

7. The use of a vector as claimed in claims 5 and 6 in the transformation or transfection of a prokaryotic or eukaryotic host.

8. A host cell suitable for the transformation of vector as claimed in claims 5 and 6.

9. An antibody, an affibody, or a derivative thereof which binds to one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, as claimed in any one of claims 1 to 3.

10. An immunogenic composition comprising one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, or nucleic acid sequences as claimed in any one or more of claims 1-3 and claim 4.

11. An immunogenic composition as claimed in claim 10 which is a vaccine.

12. Use of an immunogenic composition as a claimed in claim 10 in the preparation of a medicament for the treatment or prophylaxis of *Group B Streptococcus* infection.

13. A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one antibody, affibody, or a derivative thereof, as described herein.

14. A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one protein, polypeptide, peptide, fragments or derivatives as described herein.

15. A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one nucleic acid molecule as described herein.

16. A kit for the detection of *Group B Streptococcus* comprising at least one antibody, affibody, or derivatives thereof as claimed in claim 9.

17. A kit for the detection of *Group B Streptococcus* comprising at least one *Group B Streptococcus* protein, polypeptide, peptide, fragment or derivative thereof as claimed in claims 1 to 3.

18. A kit for the detection of *Group B Streptococcus* comprising at least one nucleic acid molecule as claimed in claim 4.

19. A method of screening for DNA encoding bacterial cell envelope associated or surface antigens in gram positive bacteria comprising the steps of:

- combining a reporter vector including the nucleotide sequence encoding the mature from of the staphylcoccus nuclease gene and an upstream promoter region with DNA from a gram positive bacteria.
- transforming the resultant vector into *Lactococcus lactis* cells.
- assaying for the secretion of staphlycoccus nuclease protein in the transformed cells.

20. A method as claimed in claim 19 wherein the reporter vector is one of the pTREP1-*nuc* vectors shown in figure 4.

21. A method as claimed in claim 19 or claim 20 wherein the gram positive bacteria is *Group B Streptococcus*, *Streptococcus Pneumoniae*, *Staphylcoccus aureus* or *pathogenic group A streptococci.*

22. A vector as shown in figure 4 for use in screening for DNA encoding bacterial cell envelope associated or secreted antigens in gram positive bacteria.

23. A method of determining whether a protein, polypeptide, peptide, fragment or derivative thereof as claimed in claims 1 to 3 represents a potential anti-microbial target which comprises inactivating said protein and determining whether *Group B Streptococcus* is still viable.

FIG. 1

ID-1

Clone 4

ATGGAAAAAAATACTTGGAAAAAATTACTTGTTAGTACTGCTGCTCTTTCAGTAGT
TGCAGGAGGAGCAATTGCTGCTACTCACTCTAACTCAGTTGATGCTGCTTCAAAAA
AAACTATCAAACTTTGGGTCCCAACAGATTCAAAAGCGTCTTATAAAGCAATTGTT
AAAAAATTCGAGAAGGAAAACAAAGGCGTTACTGTAAAAATGATTGAGTCTAATG
ACTCCAAAGCTCAAGAAAACGTAAAAAAAGACCCAAGCAAGGCAGCCGATGTATT
CTCACTTCCACATGACCAACTTGGTCAATTAGTAGAATCTGGTGTTATCCAAGAAA
TTCCAGAGCAATACTCAAAAGAAATTGCTAAAAACGACACTAAACAATCACTTAC
TGGTGCACAATATAAAGGGAAAACTTATGCATTCCCATTTGGTATTGAATCTCAAG
TTCTTTATTATAATAAAACAAAGTTAACTGCTGACGACGTTAAATCATACGAAACA
ATTACAAGCAAAGGGAAATTCGGTCAACAGCTTAAAGCAGCTAACTCATATGTAA
CAGGTCCTCTTTTCCTTTCTGTAGGCGACACTTTATTTGGTAAATCTGGTGAAGATG
CTAAAGGCACTAACTGGGGTAATGAAGCAGGTGTTTCTGTCCTTAAATGGATTGCA
GATCAAAAGAAAAATGATGGTTTTGTCAACTTGACAGCTGAAAATACAATGTCTAA
ATTTGGCGATGGTTCTGTTCATGCTTTTGAAAGTGGACCATGGGATTACGACGCTG
CTAAAAAAGCTGTCGGTGAAGATAAAATCGGTGTTGCTGTTTACCCAACAATGAAA
ATCGGTGACAAAGAAGTTCAACAAAAAGCATTCTTGGGCGTTAAACTTTATGCCGT
TAACCAAGCACCTGCTGGTTCAAACACTAAACGAATCTCAGCTAGCTACAAACTCG
CTGCATATCTAACTAATGCTGAAAGTCAAAAAATTCAATTCGAAAAACGTCATATC
GTTCCTGCTAACTCATCAATTCAATCTTCTGATAGCGTCCAAAAAGATGAACTTGC
AAAAGCAGTTATCGAAATGGGTAGCTCAGATAAATATACAACGGTTATGCCTAAG
TTGAGTCAAATGTCAACATTCTGGACAGAAAGTGCTGCTATTCTTAGCGATACTTA
CAGTGGTAAAATCAAATCTAGCGATTACCTTAAACGTCTAAAACAATTCGATAAAG
ACATCGCTAAAACAAAATAG

MEKNTWKKLLVSTAALSVVAGGAIAATHSNSVDAASKKTIKLWVPTDSKASYKAIVK
KFEKENKGVTVKMIESNDSKAQENVKKDPSKAADVFSLPHDQLGQLVESGVIQEIPEQ
YSKEIAKNDTKQSLTGAQYKGKTYAFPFGIESQVLYYNKTKLTADDVKSYETITSKGK
FGQQLKAANSYVTGPLFLSVGDTLFGKSGEDAKGTNWGNEAGVSVLKWIADQKKND
GFVNLTAENTMSKFGDGSVHAFESGPWDYDAAKKAVGEDKIGVAVYPTMKIGDKEV
QQKAFLGVKLYAVNQAPAGSNTKRISASYKLAAYLTNAESQKIQFEKRHIVPANSSIQS
SDSVQKDELAKAVIEMGSSDKYTTVMPKLSQMSTFWTESAAILSDTYSGKIKSSDY
LKRLKQFDKDIAKTKZ

ID-2

Clone 5

ATGTCAAAACAAAAAGTAACGGCAACTTTGTTGTTATCCACTTTAGTCTTATCGCT
ATCATCACCTTTAGTGACCTTAGCAGAAACTATTAATCCAGAAACAAGCCTGACAA
TGGCAACAGCATCAACAGAAAGTTCTTCTGAAGCAGAGAAACAGGAAAAAAACACA
ACCTACAGATTCAGAAACTGCTTCACCTTCAGCCGAAGGAAGTATCTCAACAGAA
AAAACAGAGATTGGTACGACAGAGACATCATCAAGCAATGAATCATCATCAAGTT
CATCACATCAATCTTCTTCCAACGAAGATGCTAAAACATCTGATTCTGCTTCAACA
GCATCTACTCCTAGCACTAATACTACAAACAGTAGTCAAGCAGACAGTAAGCCAG
GTCAATCAACAAAGACTGAATTAAAACCTGAGCCTACCTTACCATTAGTAGAGCCT
AAAATAACTCCCGCTCCGTCTCAGATAGAAAGTGTTCAGACAAATCAGAATGCTTC
TGTTCCTGCTTTATCCTTTGATGATAACTTATTATCAACACCGATTTCACCAGTGAC
AGCAACGCCATTCTACGTAGAACACTGGTCTGGTCAGGATGCCTACTCTCACTATT
TATTGTCACATCGTTACGGTATCAAAGCTGAACAATTAGATGGGTACTTAAAATCT
TTAGGGATTCAATATGATTCTAATCGTATCAATGGTGCTAAGTTATTACAATGGGA
AAAAGATAGTGGTTTAGATGTCCGTGCTATTGTAGCTATTGCTGTCCTTGAAAGTTC
ATTGGGAACTCAAGGAGTGGCTAAAATGCCAGGTGCTAATATGTTTGGTTATGGTG
CCTTTGATCATGACTCTAGCCATGCTAGTGCTTATAATGATGAAGAAGCAATTATG
TTGTTGACAAAAAATACAATTATTAAAAACAACAACTCTAGCTTTGAAATCCAAGA
TTTGAAAGCACAGAAATTATCTTCTGGACAACTTAATACAGTTACTGAGGGTGGTG
TTTATTATACAGATAACTCTGGAACTGGTAAACGTCGTGCCCAGATTATGGAAGAT
TTAGACCGCTGGATTGATCAACATGGAGGGACACCAGAAATTCCTGCTGCCTTGAA
AGCTTTATCGACAGCAAGTTTAGCAGATTTACCAAGTGGTTTTAGCTTATCAACAG
CGGTTAACACAGCTAGCTATATTGCATCAACTTATCCATGGGGTGAATGTACATGG
TATGTCTTTAACCGCGCTAAAGAGTTAGGTTATACATTTGATCCATTTATGGGTAAT
GGTGGAGATTGGCAACATAAGGCTGGCTTTGAAACAACACATTCACCAAAAGTAG
GCTATGCTGTATCATTTTCACCAGGACAAGCTGGTGCTGATGGCACTTACGGTCAC
GTAGCTATTGTTGAAGAAGTTAAAAAAGATGGTTCAGTTCTCATTTCAGAATCTAA
TGCAATGGGACGTGGTATTGTCTCTTACCGTACTTTTAGTTCAGCACAAGCTGCAC
AATTAACTTATGGTATTGGCCATAAATAA

MSKQKVTATLLLSTLVLSLSSPLVTLAETINPETSLTMATASTESSSEAEKQEKTQPTDS
ETASPSAEGSISTEKTEIGTTETSSSNESSSSSSHQSSSNEDAKTSDSASTASTPSTNTTNS
SQADSKPGQSTKTELKPEPTLPLVEPKITPAPSQIESVQTNQNASVPALSFDDNLLSTPIS
PVTATPFYVEHWSGQDAYSHYLLSHRYGIKAEQLDGYLKSLGIQYDSNRINGAKLLQ
WEKDSGLDVRAIVAIAVLESSLGTQGVAKMPGANMFGYGAFDHDSSHASAYNDEEAI
MLLTKNTIIKNNNSSFEIQDLKAQKLSSGQLNTVTEGGVYYTDNSGTGKRRAQIMEDL
DRWIDQHGGTPEIPAALKALSTASLADLPSGFSLSTAVNTASYIASTYPWGECTWYVF
NRAKELGYTFDPFMGNGGDWQHKAGFETTHSPKVGYAVSFSPGQAGADGTYGHVAI
VEEVKKDGSVLISESNAMGRGIVSYRTFSSAQAAQLTYGIGHKZ

FIG. 1CONT'D

ID-3

Clone 6

GTGCATATGTTACAAAACATTGGACAAACAGGCATTCAAGCAACTCGAATTGCTTT
AGGTTGTATGAGAATGAGTGACTTGAAAGGAAAACAAGCTGAAGAAGTAGTTGGA
ACAGCATTAGATTTGGGTATTATAAATAATAAAGTGCAAGAAAGTGTCTCTGGCGT
CAAAGTGACTAAATCATTGTGTTATCAAGAACAAGAAATTGCTTCTTTTCAAGAGA
TTAATCAGATGACTTTCGTGAAGAACATGCGGACCATGACTTATGATGTCATGTTT
GATCCTTTAGTTCTTCTTTTTATAGGTGCCTCCTACGTATTAACATTGGCTATGGGA
GCTTTTATGATTTCAAAAGGTCAAGTTACTGTTGGTGACTTGGTAACATTTGTGACG
TATTTAGATATGTTGGTATGGCCCTTGATGGCGATTGGTTTCTTGTTCAATATGGTA
CAGCGTGGTAGTGTTTCTTATAACCGTATTAATAGTCTACTTGAGCAAGAATCGGA
TATAACTGATCCTTTAAATCCTATCAAACCTGTTGTCAATGGAACATTAAGATA
TGATATTGATTTCTTTAGATACGACAATGAGGAAACCTTAGCCGATATTCATTTCAC
CTTAGAAAAAGGTCAAACCTTAGGTTTGGTAGGTCAAACGGGATCAGGGAAGACA
AGTCTTATTAAGTTATTGCTACGTGAACATGATGTGACTCAGGGGAAAATTACTTT
AAATAAACATGATATACGTGATTATCGATTGTCTGAGTTACGTCAACTAATCGGTT
ATGTTCCTCAAGATCAGTTTTTATTTGCTACCAGTATTTTAGAAAATGTTCGCTTTG
GAAATCCAACTCTATCTATCAATGCTGTCAAAGAAGCAACTAAATTGGCACATGTT
TACGATGACATTGAACAGATGCCAGCAGGATTTGAGACTCTAATTGGAGAAAAAG
GAGTCTCATTATCTGGTGGACAAAAACAAAGGATTGCGATGAGTCGTGCCATGATT
TTAGATCCAGATATTCTTATTTTGGATGATTCTCTATCAGCAGTGGACGCTAAAACG
GAACATGCTATTGTTGAGAATCTTAAAACGAATCGTCAAGGGAAATCGACTATTA
TTTCAGCACATCGTTTATCAGCTGTTGTGCACGCAGACCTTATCTTAGTTATGCGAG
ACGGCAGAGTCATTGAGCGAGGTCAACATCAAGAGTTGCTAAATAAAGGTGGTTG
GTATGCTGAAACGTATGCCTCACAGCAATTAGAAATGGAGGAAGCATTTGATGAA
GTCTAA

MHMLQNIGQTGIQATRIALGCMRMSDLKGKQAEEVVGTALDLGIINNKVQESVSGVK
VTKSLCYQEQEIASFQEINQMTFVKNMRTMTYDVMFDPLVLLFIGASYVLTLAMGAF
MISKGQVTVGDLVTFVTYLDMLVWPLMAIGFLFNMVQRGSVSYNRINSLLEQESDITD
PLNPIKPVVNGTLRYDIDFFRYDNEETLADIHFTLEKGQTLGLVGQTGSGKTSLIKLLLR
EHDVTQGKITLNKHDIRDYRLSELRQLIGYVPQDQFLFATSILENVRFGNPTLSINAVKE
ATKLAHVYDDIEQMPAGFETLIGEKGVSLSGGQKQRIAMSRAMILDPDILILDDSLSAV
DAKTEHAIVENLKTNRQGKSTIISAHRLSAVVHADLILVMRDGRVIERGQHQELLNKG
GWYAETYASQQLEMEEAFDEVZ

ID-4

Clone 6b

TTGATGAAGTCTAATCAATGGCAAGTCTTTAAGAGATTAATCTCCTATTTACGCCCT
TATAAATGGTTTACAGTATTAGCTCTATCTCTCTTATTGTTGACGACTGTTGTTAAA

FIG. 1 CONT'D

AATATTATTCCTTTAATTGCTTCACATTTTATTGATCACTATCTGACAAATGTTAAT
CAAACAGCAGTTCTTATTTTAGTGGGATATTATTCAATGTATGTCTTGCAGACCTTA
ATTCAATATTTTGGGAATCTCTTTTTTGCGCGTGTTTCTTATAGTATTGTTAGAGAT
ATTCGTAGAGATGCTTTTGCTAATATGGAAAGGCTAGGCATGTCTTATTTTGATAG
GACACCGGCAGGATCTATTGTGTCACGTATTACTAATGATACTGAAGCAATATCTG
ATATGTTTTCGGGTATTTTATCAAGTTTTATCTCGGCGATATTTATTTTTACAGTTAC
TCTGTACACTATGTTGATGCTAGACATTAAACTAACAGGACTCGTCGCTCTTTTGTT
ACCTGTTATCTTTATATTAGTGAATGTCTATCGGAAAAAATCAGTCACTGTCATTGC
TAAAACGAGAAGTTTACTTAGTGATATCAACAGTAAATTATCAGGAAGTATTGAAG
GAATTCGCATTGTACAGGCTTTTGGTCAAGAAGAGCGCTTGAAGACTGAATTTGAG
GAAATTAACAAAGAGCATGTTGTGTATGCCAATCGTTCTATGGCTCTTGATAGTCT
CTTCTTAAGACCGGCGATGTCTCTTTTAAAACTCCTAGCATATGCTGTTCTTATGTC
TTATTTTGGATTTACAGGAGTTAAAGGAGGTCTTACGGCAGGATTAATGTATGCTT
TTATTCAGTACGTTAATCGTCTATTTGACCCTTTAATTGAAGTAACGCAAAATTTTT
CAACCTTACAAACATCAATGGTATCAGCAGGGCGTGTGTTTGATCTGATTGAT
GAAACAGGTTTTGAACCAAGCCAAAAAAATACAGAAGCT

MKSNQWQVFKRLISYLRPYKWFTVLALSLLLLTTVVKNIIPLIASHFIDHYLTNVNQTA
VLILVGYYSMYVLQTLIQYFGNLFFARVSYSIVRDIRRDAFANMERLGMSYFDRTPAG
SIVSRITNDTEAISDMFSGILSSFISAIFIFTVTLYTMLMLDIKLTGLVALLLPVIFILVNVY
RKKSVTVIAKTRSLLSDINSKLSGSIEGIRIVQAFGQEERLKTEFEEINKEHVVYANRSM
ALDSLFLRPAMSLLKLLAYAVLMSYFGFTGVKGGLTAGLMYAFIQYVNRLFDPLIEVT
QNFSTLQTSMVSAGRVFDLIDETGFEPSQKNTEA

ID-5

Clone 7

ATGAAAAGAAAAGACTTATTTGGTGATAAACAAACTCAATACACGAT
TAGAAAGTTAAGTGTTGGAGTAGCTTCAGTTGCAACAGGGGTATGTA
TTTTTCTTCATAGTCCACAGGTATTTGCTGAAGAAGTAAGTGTTTCTC
CTGCAACTACAGCGATTGCAAAGTCGAATATTAATCAGGTTGACAAC
CGGCAATCTACTAATTTAAAAGATGACATAAACTCAAACTCTGAGAC
GGTTGTGACACCCTCAGATATGCCGGATACCAAGCAATTAGTATCAG
ATGAAACTGACACTCAAAAAGGAGTGACAGAGCCGGATAAGGCGAC
AAGCCTGCTTGAAGAAAATAAAGGTCCTGTTTCAGATAAAAATACCT
TAGATTTAAAAGTGGCACCATCTACATTGCAAAATACTCCCGACAAA
ACTTCTCAAGCTATAGGTGCTCCAAGTCCGACCTTGAAAGTTGCTAAT
CAAGCTCCACAGATTGAAAATGGTTACTTTAGGTTACATCTTAAAGA
ATTGCCTCAAGGTCATCCTGTAGAAAGCACTGGGCTTTGGATATGGG
GAGATGTTGATCAACCGTCTAGTAATTGGCCAAATGGTGCTATCCCT
ATGACTAATGCTAAGAAAGATGATTACGGTTATTATGTTGATTTTAA
ATTATCTGAAAAACAACGAAAACAAATATCTTTTTTAATTAATAACA
AAGCAGGAACAAATTTAAGCGGCGATCATCATATTCCATTATTACGA

FIG. 1 CONT'D

CCTGAGATGAACCAAGTTTGGATTGATGAAAAGTACGGTATACATAC
TTATCAGCCCCTCAAAGAAGGGTATGTCCGTATTAACTATTTGAGTTC
ATCTGGTAACTATGACCACTTATCAGCATGGCTCTTTAAAGATGTTGC
AACCCCCTCAACAACTTGGCCAGATGGTAGTAATTTTGTGAATCAAG
GACTATATGGAAGGTATATTGATGTACCACTGAAAACTAATGCCAAA
GAGATTGGTTTCTAATCTTAGATGAAAGTAAGACAGGAGATGCAGT
GAAAGTTCAACCCAACGACTATGTTTTTAGAGATTTAGCTAACCATA
ACCAAATTTTTGTAAAAGATAAGGATCCAAAGGTTTATAATAATCCT
TATTACATTGATCAAGTGCAGCTAAAGGATGCTCAACAAACTGATTT
AACAAGTATTCAAGCAAGTTTTACAACTCTAGATGGGGTAGATAAAA
CTGAAATTTTAAAAGAATTGAAAGTGACAGATAAAAATCAAAATGCT
ATACAAATTTCTGATATCACTCTCGATACTAGTAAATCTCTTTTAATA
ATCAAAGGCGACTTTAATCCTAAACAAGGTCATTTCAATATATCTTAT
AATGGTAACAATGTCACGACAAGGCAATCTTGGGAATTTAAAGACCA
ACTTTATGCTTATAGTGGAAATTTAGGTGCAGTTCTCAATCAAGATGG
TTCAAAAGTTGAAGCCAGCCTCTGGTCACCGAGTGCTGATAGTGTCA
CTATGATTATTTATGACAAAGATAATCAAAACAGGGTTGTAGCGACT
ACCCCCCTTGTGAAAAATAATAAAGGTGTTTGGCAGACGATACTTGA
TACTAAATTAGGTATTAAAAACTATACTGGTTACTATTATCTTTACGA
AATAAAAAGAGGTAAGGATAAGGTTAAGATTTTAGATCCTTATGCAA
AGTCATTAGCAGAGTGGGATAGTAATACTGTTAATGACGATATAAAA
ACGGCTAAAGCAGCTTTTGTAAATCCAAGTCAACTTGGACCTAAAAA
TTTAAGTTTTGCTAAAATTGCTAATTTTAAAGGAAAACAAGATGCTGT
TATATACGAAGCACATGTAAGAGACTTCACTTCTGATCAATCTTTGG
ACGGAAAATTAAAAAATCAACTTGGTACCTTTGCAGCCTTTTCAGAG
AAACTAGATTATTTACAGAAATTAGGAGTTACACACATTCAGCTTTT
ACCGGTATTGAGTTATTTTTATGTTAATGAAATGGATAAGTCACGCTC
AACAGCTTACACTTCCTCAGACAATAATTACAATTGGGGCTATGACC
CACAGAGCTATTTTGCTCTTTCTGGAATGTATTCAGAGAAACCAAAA
GATCCATCAGCACGTATCGCCGAATTAAAACAATTAATACATGATAT
TCATAAACGTGGCATGGGGGTTATACTTGATGTCGTCTATAATCACA
CTGCAAAAACTTATCTCTTTGAGGATATAGAACCTAATTATTATCACT
TTATGAATGAAGATGGTTCACCAAGAGAAAGTTTTGGAGGGGGACGT
TTAGGAACCACTCATGCAATGAGTCGTCGTGTTTTGGTTGATTCCATT
AAATATCTTACAAGTGAATTTAAAGTTGATGGTTTCCGTTTTGATATG
ATGGGAGATCATGATGCGGCTGCGATTGAATTAGCTTATAAAGAAGC
TAAAGCTATTAATCCTAATATGATTATGATTGGTGAGGGCTGGAGAA
CATTCCAAGGCGATCAAGGTAAGCCGGTTAAACCAGCTGACCAAGAT
TGGATGAAGTCAACCGATACAGTTGGCGTCTTTTCAGATGATATTCGT
AATAGCTTGAAATCTGGTTTTCCAAATGAAGGTACTCCAGCTTTCATC
ACAGGTGGCCCACAATCTTTACAAGGTATTTTTAAAAATATCAAAGC
ACAACCTGGGAATTTTGAAGCAGATTCGCCAGGAGATGTGGTGCAGT
ATATTGCTGCACATGATAACCTTACCTTGCATGATGTGATTGCAAAAT
CAATTAATAAAGACCCTAAGGTAGCTGAAGAAGATATTCATAGACGT

FIG. 1 CONT'D

CTGCGTTTAGGAAATGTAATGATTTTAACATCTCAAGGGACAGCATT
CATTCATTCTGGTCAAGAGTATGGTCGTACGAAGCGTTTACTTAACCC
TGATTACATGACAAAAGTTTCAGATGACAAATTGCCTAATAAAGCAA
CACTTATTGAAGCTGTTAAAGAATACCCATATTTTATTCATGATTCAT
ATGATTCTTCAGATGCCATTAATCATTTTGATTGGGCAGCAGCCACAG
ATAATAACAAACACCCAATTTCAACGAAAACACAGGCCTATACAGCA
GGTTTAATCACATTAAGGCGTTCAACAGATGCTTTCCGGAAATTGAG
CAAAGCAGAAATTGATCGTGAGGTTAGCTTGATTACAGAGGTAGGTC
AAGGTGATATTAAAGAAAAAGATTTGGTTATTGCTTACCAAACAATA
GATTCTAAAGGCGATATTTACGCAGTATTTGTTAATGCTGATAGTAA
AGCTAGAAACGTTTTACTAGGTGAAAAATATAAACACCTTTTAAAAG
GGCAAGTAATTGTTGATGCTGATCAAGCGGGGATTAAACCAATCTCA
ACTCCTAGAGGTGTTCATTTTGAAAAAGATAGTTTGCTGATTGATCCA
TTAACAGCAATTGTGATTAAAGTTGGCAAAGTTGCTCCTAGCCCTAA
GGAGGAATTGCAAGCAGATTATCCCAAAACACAATCTTTCAAGGGAT
CTAAAACGGTAGAAAAAGTAAATAGAATAGCTAATAAGACCTCAAT
AACTCCTGTAGTTTCTAATAAGACCGATTCATATCTGACAAATGAAG
CTAATTTGCCAAAAACTGGAGATAAGTCATCAAAAATACTAAGTGTA
GTAGGAATAAGCATTCTAGCAAGTCTACTTGCTCTACTAGGTCTCTCT
TTAAAGAGGAATCGCACTTAA

MKRKDLFGDKQTQYTIRKLSVGVASVATGVCIFLHSPQVFAEEVSVSPA
TTAIAKSNINQVDNRQSTNLKDDINSNSETVVTPSDMPDTKQLVSDETDT
QKGVTEPDKATSLLEENKGPVSDKNTLDLKVAPSTLQNTPDKTSQAIGA
PSPTLKVANQAPQIENGYFRLHLKELPQGHPVESTGLWIWGDVDQPSSN
WPNGAIPMTNAKKDDYGYYVDFKLSEKQRKQISFLINNKAGTNLSGDH
HIPLLRPEMNQVWIDEKYGIHTYQPLKEGYVRINYLSSSGNYDHLSAWL
FKDVATPSTTWPDGSNFVNQGLYGRYIDVPLKTNAKEIGFLILDESKTGD
AVKVQPNDYVFRDLANHNQIFVKDKDPKVYNNPYYIDQVQLKDAQQT
DLTSIQASFTTLDGVDKTEILKELKVTDKNQNAIQISDITLDTSKSLLIIKG
DFNPKQGHFNISYNGNNVTTRQSWEFKDQLYAYSGNLGAVLNQDGSKV
EASLWSPSADSVTMIIYDKDNQNRVVATTPLVKNNKGVWQTILDTKLGI
KNYTGYYYLYEIKRGKDKVKILDPYAKSLAEWDSNTVNDDIKTAKAAF
VNPSQLGPKNLSFAKIANFKGKQDAVIYEAHVRDFTSDQSLDGKLKNQL
GTFAAFSEKLDYLQKLGVTHIQLLPVLSYFYVNEMDKSRSTAYTSSDNN
YNWGYDPQSYFALSGMYSEKPKDPSARIAELKQLIHDIHKRGMGVILDV
VYNHTAKTYLFEDIEPNYYHFMNEDGSPRESFGGGRLGTTHAMSRRVL
VDSIKYLTSEFKVDGFRFDMMGDHDAAAIELAYKEAKAINPNMIMIGEG
WRTFQGDQGKPVKPADQDWMKSTDTVGVFSDDIRNSLKSGFPNEGTPA
FITGGPQSLQGIFKNIKAQPGNFEADSPGDVVQYIAAHDNLTLHDVIAKSI
NKDPKVAEEDIHRRLRLGNVMILTSQGTAFIHSGQEYGRTKRLLNPDYM
TKVSDDKLPNKATLIEAVKEYPYFIHDSYDSSDAINHFDWAAATDNNKH
PISTKTQAYTAGLITLRRSTDAFRKLSKAEIDREVSLITEVGQGDIKEKDL

FIG. 1CONT'D

VIAYQTIDSKGDIYAVFVNADSKARNVLLGEKYKHLLKGQVIVDADQA
GIKPISTPRGVHFEKDSLLIDPLTAIVIKVGKVAPSPKEELQADYPKTQSFK
GSKTVEKVNRIANKTSITPVVSNKTDSYLTNEANLPKTGDKSSKILSVVG
ISILASLLALLGLSLKRNRT*

ID-6

Clone 9

ATGAAAAAAGTTTTTTTCTCATGGCTATGGTTGTGAGTTTAGTAATGATAGCAGG
GTGTGATAAGTCAGCAAACCCCAAACAGCCTACGCAAGGCATGTCAGTTGTAACC
AGCTTTTACCCAATGTATGCGATGACAAAAGAAGTATCTGGAGACCTAAATGATGT
GAGGATGATCCAATCAGGTGCAGGCATTCATTCCTTTGAACCGTCTGTAAATGATG
TGGCAGCTATTTATGACGCGGATTTGTTTGTTTACCAATCACATACCTTAGAAGCTT
GGGCAAGGGATCTAGACCCTAATTTAAAAAAATCAAAGGTTAATGTGTTTGAAGC
GTCAAAACCTCTGACACTAGATAGAGTCAAAGGGCTAGAAGATATGGAAGTCACA
CAAGGCATTGACCCTGCGACACTTTATGACCCACATACCTGGACGGATCCCGTTTT
AGCTGGTGAGGAAGCTGTTAATATCGCTAAAGAGCTAGGACATTTGGATCCTAAAC
ACAAAGACAGTTACACTAAAAAGGCTAAGGCTTTCAAAAAAGAAGCAGAGCAACT
AACTGAAGAATACACTCAAAAATTTAAAAAGGTGCGCTCAAAAACATTTGTGACG
CAACACACGGCATTTTCTTATCTGGCTAAACGATTCGGCTTGAAACAACTTGGTAT
CTCGGGTATTTCTCCAGAGCAAGAGCCCTCTCCTCGCCAATTGAAAGAAATTCAAG
ACTTTGTTAAAGAATACAACGTCAAGACTATTTTTGCAGAAGACAACGTCAACCCC
AAAATTGCTCATGCTATTGCGAAATCAACAGGAGCTAAAGTAAAGACATTAAGTC
CACTTGAAGCTGCTCCAAGCGGAAACAAGACATATCTAGAAAATCTTAGAGCAAA
TTTGGAAGTGCTCTATCAACAGTTGAAGTAA

MKKVFFLMAMVVSLVMIAGCDKSANPKQPTQGMSVVTSFYPMYAMTKEVSGDLND
VRMIQSGAGIHSFEPSVNDVAAIYDADLFVYQSHTLEAWARDLDPNLKKSKVNVFEAS
KPLTLDRVKGLEDMEVTQGIDPATLYDPHTWTDPVLAGEEAVNIAKELGHLDPKHKD
SYTKKAKAFKKEAEQLTEEYTQKFKKVRSKTFVTQHTAFSYLAKRFGLKQLGISGISPE
QEPSPRQLKEIQDFVKEYNVKTIFAEDNVNPKIAHAIAKSTGAKVKTLSPLEAAPSGNK
TYLENLRANLEVLYQQLK*

ID-7

Clone 15

TTGTTCAATAAAATAGGTTTTAGAACTTGGAAATCAGGAAAGCTTTG
GCTTTATATGGGAGTGCTAGGATCAACTATTATTTTAGGATCAAGTCC
TGTATCTGCTATGGATAGTGTTGGAAATCAAAGTCAAGGTAATGTTTT
AGAGCGTCGCCAACGTGATGCGGAAAACAAAAGTCAGGGTAATGTT
TTAGAGCGTCGCCAACGTGATGCGGAAAACAAGAGCCAAGGCAATG
TTTTAGAGCGTCGTCAACGCGATGTTGAGAATAAGAGCCAAGGCAAT

FIG. 1CONT'D

GTTTTAGAGCGTCGTCAACGTGATGCGGAAAACAAAAGTCAGGGCA
ATGTTCTAGAGCGCCGCCAACGTGATGCGGATAACAAGAGCCAAGTA
GGTCAACTTATAGGGAAAAATCCACTTTTTTCAAAGCCAACTGTATCT
AGAGAAAATAATCACTCTAGTCAAGGTGACTCTAACAAACAGTCATT
CTCTAAAAAAGTATCTCAGGTTACTAATGTAGCTAATAGACCGATGT
TAACTAATAATTCTAGAACAATTTCAGTGATAAATAAATTACCTAAA
ACAGGTGGTGATCAAAATGTCATTTTTAAACTTGTAGGTTTTGGTTTA
ATTTTGTTAACAAGTCGCTGCGGTTTGAGACGCAATGAAAATTAA

MFNKIGFRTWKSGKLWLYMGVLGSTIILGSSPVSAMDSVGNQSQGNVL
ERRQRDAENKSQGNVLERRQRDAENKSQGNVLERRQRDVENKSQGNV
LERRQRDAENKSQGNVLERRQRDADNKSQVGQLIGKNPLFSKPTVSREN
NHSSQGDSNKQSFSKKVSQVTNVANRPMLTNNSRTISVINKLPKTGGDQ
NVIFKLVGFGLILLTSRCGLRRNEN*

ID-8

Clone 17

ATGACAAAAAAACTTATTATTGCTATATTAGCACTATGCACTATCTTAACCACTTCT
CAAGCTGTTTTAGCTAAAGAAAAATCACAAACTGTTACCATAAAAAACAACTATTC
GGTCTATATTAAAAAAGAAAAAAGAGACAAGCCGGATAATAAAAAGCAAATCAG
CGAGACACTTAAAGTTCCTTTAAAACCCAAAAAAGTAGTTGTTTTTGATATGGGAG
CTTTGGATACTATCACAGCTTTAGGAGCTGAAAAATCTGTTATTGGTATCCCGAAG
GCTAAAAATGCTCTAAGTTTATTGCCCAATAACGTCAAATCTGTTTATAAAGCTAA
GAGATACCAAGACGTAGGAAGTCTCTTCGAACCAAACTTTGAAGCTATTGCTCGTA
TGCAACCTGATGTGGTTTTCCTAGGAGCACGTATGGCTTCTGTTGATAATATTGAA
AAATTAAAGGAGGCTGCACCTAAAGCAGCATTAGTATATGCTGGAGTCGACTCAA
AAAAAGTATTTGACAAAGGAGTTGCTGAGCGTGTCACAATGTTAGGGAAAATCTTC
GACCAAAATAAAAAGGCAAAAACCTTTAATAAAGATATCGCACAAGCTGTTCTTA
AATTGCAGAAAACTATTGAGAAAAAAGGTAAACCTACAGCTCTATTTGTAATGGC
AAACAGCGGTGAACTTTTAACTCAATCACCTTCTGGTCGTTTTGGTTGGATTTTCTC
TGTAGGTGGATTTAAAGCAGTCAATGAAAATGAAAAACTAAGTTCACATGGTACTC
CCGTATCTTATGAATACATCGCTGAAAAAAATCCTAACTATCTCTTTGTTTTAGATC
GTGGAGCGACTATTGGACAAGGAGCTTCATCAAAAGAACTTTTTAATAACGATGTT
ATTAAAGCAACTGATGCTGTCAAAAACAAACGTGTTCATGAGGTAGATGGAAAAG
ATTGGTATATCAATTCAGGCGGAAGCCGAGTAACACTCCGTATGATTAAAGATGTA
CAGAACTTTGTTGATAATCGTTAA

MTKKLIIAILALCTILTTSQAVLAKEKSQTVTIKNNYSVYIKKEKRDKPDN
KKQISETLKVPLKPKKVVVFDMGALDTITALGAEKSVIGIPKAKNALSLL
PNNVKSVYKAKRYQDVGSLFEPNFEAIARMQPDVVFLGARMASVDNIE
KLKEAAPKAALVYAGVDSKKVFDKGVAERVTMLGKIFDQNKKAKTFN
KDIAQAVLKLQKTIEKKGKPTALFVMANSGELLTQSPSGRFGWIFSVGG

FIG. 1 CONT'D

FKAVNENEKLSSHGTPVSYEYIAEKNPNYLFVLDRGATIGQGASSKELFN
NDVIKATDAVKNKRVHEVDGKDWYINSGGSRVTLRMIKDVQNFVDNR
*

ID-9

Clone 18

GTGAAGAAAACATATGGTTATATCGGCTCAGTTGCTGCTATTTTACTAGCTACTCAT
ATTGGAAGTTACCAGCTTGGTAAGCATCATATGGGTCTAGCAACAAAGGACAATC
AGATTGCCTATATTGATGATAGCAAAGGTAAGGTAAAAGCCCCTAAAACAAACAA
AACGATGGATCAAATCAGTGCTGAAGAAGGCATCTCTGCTGAACAGATCGTAGTC
AAAATTACTGACCAAGGTTATGTTACCTCACACGGTGACCATTATCATTTTTACAAT
GGGAAAGTTCCTTATGATGCGATTATTAGTGAAGAGTTGTTGATGACGGATCCTAA
TTACCATTTTAAACAATCAGACGTTATCAATGAAATCTTAGACGGTTACGTTATTA
AAGTCAATGGCAACTATTATGTTTACCTCAAGCCAGGTAGTAAGCGCAAAAACATT
CGAACCAAACAACAAATTGCTGAGCAAGTAGCCAAAGGAACTAAAGAAGCTAAA
GAAAAAGGTTTAGCTCAAGTGGCCCATCTCAGTAAAGAAGAAGTTGCGGCAGTCA
ATGAAGCAAAAAGACAAGGACGCTATACTACAGACGATGGCTATATTTTTAGTCC
GACAGATATCATTGATGATTTAGGAGATGCTTATTTAGTACCTCATGGTAATCACT
ATCATTATATTCCTAAAAAAGATTTGTCTCCAAGTGAGCTAGCTGCTGCACAAGCC
TACTGGAGTCAAAAACAAGGTCGAGGTGCTAGACCGTCTGATTACCGCCCGACAC
CAGCCCCAGGTCGTAGGAAAGCCCCAATTCCTGATGTGACGCCTAACCCTGGACA
AGGTCATCAGCCAGATAACGGTGGTTATCATCCAGCGCCTCCTAGGCCAAATGATG
CGTCACAAAACAAACACCAAAGAGATGAGTTTAAAGGAAAAACCTTTAAGGAACT
TTTAGATCATCTACACCGTCTTGATTTGAAATACCGTCATGTGGAAGAAGATGGGT
TGATTTTTGAACCGACTCAAGTGATCAAATCAAACGCTTTTGGGTATGTGGTGCCT
CATGGAGATCATTATCATATTATCCCAAGAAGTCAGTTATCACCTCTTGAAATGGA
ATTAGCAGATCGATACTTAGCCGGCCAAACTGATGACAACGACTCAGGTTCAGATC
ACTCAAAACCATCAGATAAAGAAGTGACACATACCTTTCTTGGTCATCGCATCAAA
GCTTACGGAAAAGGCTTAGATGGTAAACCATATGATACGAGTGATGCTTATGTTTT
TAGTAAAGAATCCATTCATTCAGTGGATAAATCAGGAGTTACAGCTAAACACGGA
GATCATTTCCACTATATAGGATTTGGAGAACTTGAACAATATGAGTTGGATGAGGT
CGCTAACTGGGTGAAAGCAAAAGGTCAAGCTGATGAGCTTGTTGCTGCTTTGGATC
AGGAACAAGGCAAAGAAAAACCACTCTTTGACACTAAAAAAGTGAGTCGCAAAGT
AACAAAAGATGGTAAAGTGGGCTATATTATGCCAAAAGATGGCAAGGACTATTTC
TATGCTCGTTATCAACTTGATTTGACTCAGATTGCCTTTGCCGAACAAGAACTAATG
CTTAAAGATAAGAAGCATTACCGTTATGACATTGTTGATACAGGCATTGAGCCACG
ACTTGCTGTAGATGTGTCAAGTCTGCCGATGCATGCTGGTAATGCTACTTACGATA
CTGGAAGTTCGTTTGTTATCCCACATATTGATCATATCCATGTCGTTCCGTATTCAT
GGTTGACGCGCAATCAGATTGCAACAATCAAGTATGTGATGCAACACCCCGAAGT
TCGTCCGGATGTATGGTCTAAGCCAGGGCATGAAGAGTCAGGTTCGGTCATTCCAA
ATGTTACGCCTCTTGATAAACGTGCT

FIG. 1CONT'D

GGTATGCCAAACTGGCAAATTATCCATTCTGCTGAAGAAGTTCAAAAAGCCCTAGC
AGAAGGTCGTTTTGCAGCACCAGACGGCTATATTTTCGATCCACGAGATGTTTTGG
CAAAAGAAACTTTTGTATGGAAAGATGGCTCCTTTAGCATCCCAAGAGCAGATGGC
AGTTCATTGAGAACCATTAATAAATCCGATCTATCCCAAGCTGAGTGGCAACAAGC
TCAAGAGTTATTGGCAAAGAAAAATGCTGGTGATGCTACTGATACGGATAAACCT
GAAGAAAAGCAACAGGCAGATAAGAGCAATGAAAACCAACAGCCAAGTGAAGCC
AGTAAAGAAGAAAAAGAATCAGATGACTTTATAGACAGTTTACCAGACTATGGTC
TAGATAGAGCAACCCTAGAAGATCATATCAATCAATTAGCACAAAAAGCTAATAT
CGATCCTAAGTATCTCATTTTCCAACCAGAAGGTGTCCAATTTTATAATAAAAATG
GTGAATTGGTAACTTATGATATCAAGACACTTCAACAAATAAACCCTTAA

MKKTYGYIGSVAAILLATHIGSYQLGKHHMGLATKDNQIAYIDDSKGKVKAPKTNKT
MDQISAEEGISAEQIVVKITDQGYVTSHGDHYHFYNGKVPYDAIISEELLMTDPNYHFK
QSDVINEILDGYVIKVNGNYYVYLKPGSKRKNIRTKQQIAEQVAKGTKEAKEKGLAQV
AHLSKEEVAAVNEAKRQGRYTTDDGYIFSPTDIIDDLGDAYLVPHGNHYHYIPKKDLS
PSELAAAQAYWSQKQGRGARPSDYRPTPAPGRRKAPIPDVTPNPGQGHQPDNGGYHP
APPRPNDASQNKHQRDEFKGKTFKELLDHLHRLDLKYRHVEEDGLIFEPTQVIKSNAF
GYVVPHGDHYHIIPRSQLSPLEMELADRYLAGQTDDNDSGSDHSKPSDKEVTHTFLGH
RIKAYGKGLDGKPYDTSDAYVFSKESIHSVDKSGVTAKHGDHFHYIGFGELEQYELDE
VANWVKAKGQADELVAALDQEQGKEKPLFDTKKVSRKVTKDGKVGYIMPKDGKDY
FYARYQLDLTQIAFAEQELMLKDKKHYRYDIVDTGIEPRLAVDVSSLPMHAGNATYD
TGSSFVIPHIDHIHVVPYSWLTRNQIATIKYVMQHPEVRPDVWSKPGHEESGSVIPNVTP
LDKRAGMPNWQIIHSAEEVQKALAEGRFAAPDGYIFDPRDVLAKETFVWKDGSFSIPR
ADGSSLRTINKSDLSQAEWQQAQELLAKKNAGDATDTDKPEEKQQADKSNENQQPSE
ASKEEKESDDFIDSLPDYGLDRATLEDHINQLAQKANIDPKYLIFQPEGVQFYNKNGEL
VTYDIKTLQQINP*

ID-10

Clone 22

ATGATACGCCAGTTTTTAAGAGAACACTTGATTTGGTATATTTTATATATCATGATG
TTTGTCCTATTTTTTATTAGTTTCTATCTATATCATTTACCAATGCCCTATTTGTTTA
ATTCCTTAGGTTTAAATGTTATTGTTTTACTAGGAATTAGTATTTGGCAATACAGTC
GTTACAGGAAAAAAATGTTACATCTCAAATATTTTAATAGTAGTCAGGACCCCTCT
TTCGAACTTCAACCGAGTGATTACGCTTATTTTAATATTATTACACAATTAGAAGCT
AGAGAAGCGCAAAAAGTTTCTGAAACAATTGAACAAACCAATCATGTTGCACTTA
TGATAAAGATGTGGTCGCACCAAATGAAAGTTCCATTGGCAGCTATTTCATTAATG
GCCCAGACAAATCATCTCGATCCTAAGGAAGTTGAACAACAATTATTGAAATTGCA
ACATTATCTTGAAACGTTGTTAGCATTTTTGAAATTTAGACAATATCGTGACGATTT
TCGTTTTGAAGCTGTTAGCCTTAGAGAAGTAGTAGTAGAAATTATAAAATCGTATA
AGGTTATTTGTCTATCCAAAAGCTTATCTATCATAATTGAAGGCGATAATATCTGG
AAAACAGACAAAAAGTGGTTAACTTTTGCTCTTTCACAGGTGCTAGATAATGCCAT

FIG. 1CONT'D

AAAATATTCTAATCCTGAGTCAAAGATAATAATAAGCATAGGAGAAGAGAGTATT
AGAATACAAGACTACGGTATCGGCATACTCGAAGAGGATATCCCTAGACTTTTTGA
AGATGGCTTTACGGGTTACAACGGTCATGAGCACCAAAAGGCAACAGGCATGGGG
TTATATATGACAAAAGAAGTCTTATCTAGTCTGAATTTGTCCATTTCGGTGGATAGC
AAAATTAATTATGGGACTGCTGTTTCTATACATAAATAA

MIRQFLREHLIWYILYIMMFVLFFISFYLYHLPMPYLFNSLGLNVIVLLGISIWQYSRYR
KKMLHLKYFNSSQDPSFELQPSDYAYFNIITQLEAREAQKVSETIEQTNHVALMIKMW
SHQMKVPLAAISLMAQTNHLDPKEVEQQLLKLQHYLETLLAFLKFRQYRDDFRFEAV
SLREVVVEIIKSYKVICLSKSLSIIIEGDNIWKTDKKWLTFALSQVLDNAIKYSNPESKIIIS
IGEESIRIQDYGIGILEEDIPRLFEDGFTGYNGHEHQKATGMGLYMTKEVLSSLNLSISV
DSKINYGTAVSIHKZ

ID-11

Clone 23

ATGACTTATCAAAAAACAGTTGTTTTGGCTGGTGATTATTCCTACATTAGACAAATT
GAAACCACATTAAAATCTCTCTGTGTCTATCATGAGAATCTCTCAATTTTTATTTTT
AATCAAGATATTCCTCAAGAATGGTTTTTAGCTATGAAAGATAGGGTTGGACAAAC
TGGAAATCAAATTCAGGATGTAAAGCTCTTCCATGATCACTTATCCCCAAAATGGG
AAAATAAAAAGCTTAATCATATTAATTATATGACCTATGCTCGTTATTTCATACCTC
AGTACATCTCAGCTGATACAGTTTTATATCTTGACTCTGACTTAGTTGTTACTACTA
ATTTAGATAACCTCTTTCAAATTTCACTAGACAATGCATATTTAGCTGCAGTTCCAG
CTCTTTTTGGGCTTGGATATGGGTTTAATGCTGGAGTAATGGTAATTAACAACCAA
CGTTGGCGACAAGAAAATATGACTATTAAATTAATTGAAAAAAATCAAAAGGAAA
TTGAGAATGCCAACGAAGGGGATCAAACAATTCTTAATCGCATGTTTGAAAATCAG
GTAATTTATTTAGATGATACCTACAATTTTCAAATTGGTTTTGATATGGGAGCTGCT
ATCGATGGGCATAAATTTATTTTTGACATCCCAATTACCCCACTCCCAAAAATTATT
CACTACATTTCGGGAATCAAACCTTGGCAAACATTATCAAATATGAGACTCCGTGA
GGTATGGTGGCACTATAATTTACTTGAATGGTCAAGTATCATATCTAGTAAAAAAG
TATTTGGTTTAGACCACCCAATTAAAACACAAAATTATCGTCTCAATTTCCTTATTG
CTACAACTTCTGATTGTATACCATCTATCTCAGAATTAGTCACTGCCCTTCCAGATT
GTCTATTTCACATTGCATGCACCAACAGTTATGTCTGA

MTYQKTVVLAGDYSYIRQIETTLKSLCVYHENLSIFIFNQDIPQEWFLAMKDRVGQTG
NQIQDVKLFHDHLSPKWENKKLNHINYMTYARYFIPQYISADTVLYLDSDLVVTTNLD
NLFQISLDNAYLAAVPALFGLGYGFNAGVMVINNQRWRQENMTIKLIEKNQKEIENAN
EGDQTILNRMFENQVIYLDDTYNFQIGFDMGAAIDGHKFIFDIPITPLPKIIHYISGIKPW
QTLSNMRLREVWWHYNLLEWSSIISSKKVFGLDHPIKTQNYRLNFLIATTSDCIPSISEL
VTALPDCLFHIACTNSYV*

ID-12

FIG. 1CONT'D

Clone 27

GTGAAGAAAACATATTGTTATATCGGCTCAGTTGCTGCTATTTTACTAGCTACTCAT
ATTGGAAGTTACCAGCTTGGTAAGCATCATATGGGTCTAGCAACAAAGGACAATC
AGATTGCCTATATTGATGATAGCAAAGGTAAGGTAAAAGCCCCTAAAACAAACAA
AACGATGGATCAAATCAGTGCTGAAGAAGGCATCTCTGCTGAACAGATCGTAGTC
AAAATTACTGACCAAGGTTATGTTACCTCACACGGTGACCATTATCATTTTTACAAT
GGGAAAGTTCCTTATGATGCGATTATTAGTGAAGAGTTGTTGATGACGGATCCTAA
TTACCATTTTAAACAATCAGACGTTATCAATGAAATCTTAGACGGTTACGTTATTA
AAGTCAATGGCAACTATTATGTTTACCTCAAGCCAGGTAGTAAGCGCAAAAACATT
CGAACCAAACAACAAATTGCTGAGCAAGTAGCCAAAGGAACTAAAGAAGCTAAA
GAAAAAGGTTTAGCTCAAGTGGCCCATCTCAGTAAAGAAGAAGTTGCGGCAGTCA
ATGAAGCAAAAAGACAAGGACGCTATACTACAGACGATGGCTATATTTTTAGTCC
GACAGATATCATTGATGATTTAGGAGATGCTTATTTAGTACCTCATGGTAATCACT
ATCATTATATTCCTAAAAAAGATTTGTCTCCAAGTGAGCTAGCTGCTGCACAAGCC
TACTGGAGTCAAAAACAAGGTCGAGGTGCTAGACCGTCTGATTACCGCCCGACAC
CAGCCCCAGGTCGTAGGAAAGCCCCACTTCCTGATGTGACGCCTAACCCTGGACAA
GGTCATCAGCCAGATAACGGTGGTTATCATCCAGCGCCTCCTAGGCCAAATGATGC
GTCACAAAACAAACACCAAAGAGATGAGTTTAAAGGAAAAACCTTTAAGGAACTT
TTAGATCAACTACACCGTCTTGATTTGAAATACCGTCATGTGGAAGAAGATGGGTT
GATTTTTGAACCGACTCAAGTGATCAAATCAAACGCTTTTGGGTATGTGGTGCCTC
ATGGAGATCATTATCATATTATCCCAAGAAGTCAGTTATCACCTCTTGAAATGGAA
TTAGCAGATCGATACTTAACCCGGCCAAACTGA

MKKTYCYIGSVAAILLATHIGSYQLGKHHMGLATKDNQIAYIDDSKGKVKAPKTNKT
MDQISAEEGISAEQIVVKITDQGYVTSHGDHYHFYNGKVPYDAIISEELLMTDPNYHFK
QSDVINEILDGYVIKVNGNYYVYLKPGSKRKNIRTKQQIAEQVAKGTKEAKEKGLAQV
AHLSKEEVAAVNEAKRQGRYTTDDGYIFSPTDIIDDLGDAYLVPHGNHYHYIPKKDLS
PSELAAAQAYWSQKQGRGARPSDYRPTPAPGRRKAPLPDVTPNPGQGHQPDNGGYHP
APPRPNDASQNKHQRDEFKGKTFKELLDQLHRLDLKYRHVEEDGLIFEPTQVIKSNAF
GYVVPHGDHYHIIPRSQLSPLEMELADRYLTRPN*

ID-13

Clone 28

ATGGTAAATGATATATTAGAAAGAATGTATAAAGAGAATATTCCAAAATCTTACCT
TACATCCGTCCCATTAGTTATTTCTCAAAAAGGAAGAACAACCTATTCGTTTAGTAT
GACTGGTGGTCAACAAATAGATGGAGTGAAATTCACACAGATATATGAGGACTAT
ATGAAATTACTCAGTCAAGGTAAGGATATCGCAGAGTTATATCAAAAATATTCTAA
AGAAGAGTTGGCAAATCTAGGCATTAATATTTATCAATCCAATGATATAGAAAGG
ACTGAGGAAAGAACTTTTGATGAAATTATCAGTTGGGTTTCCAACCCTTATGCAAC
AAGACCAATTCAAGAAAGGCACACTATTCAATTAGAGCCAACAAGATTTTCACTA

FIG. 1CONT'D

GAGGATAAGAAAAGAATTGAAGAAGCTGCAGCTCAAGGACTAAGCGAAATCGAC
CTTATTGATTTAGTTGACCTATATGATATTAATTTAGACAATACAAGCGTCAATCGC
CATATTGTGGGGTTATTGACTAATAACACCCAAGTAACATACTATTTCCAAGAACA
ATTAAATAAGGAGTTGCTGTCAATGGCTCACGCTTTAGATAACGTACAACAGGCCT
TTATTAAATTATTAAGTGAAGAGGAGATACGAAAATTTGCTCTTTAA

MVNDILERMYKENIPKSYLTSVPLVISQKGRTTYSFSMTGGQQIDGVKFTQIYEDYMK
LLSQGKDIAELYQKYSKEELANLGINIYQSNDIERTEERTFDEIISWVSNPYATRPIQERH
TIQLEPTRFSLEDKKRIEEAAAQGLSEIDLIDLVDLYDINLDNTSVNRHIVGLLTNNTQV
TYYFQEQLNKELLSMAHALDNVQQAFIKLLSEEEIRKFAL*

ID-14

Clone 31

ATGAATAAAAGAAGAAAATTATCAAAATTGAATGTAAAAAAACAACATTTAGCTT
ATGGAGCTATCACTTTAGTAGCCCTTTTTTCATGTATTTTGGCTGTAACGGTCATCT
TTAAAAGTTCACAAGTTACTACTGAATCTTTGTCAAAAGCAGATAAAGTTCGCGTA
GCCAAAAAATCAAAAATGACTAAGGCGACATCTAAATCAAAAGTAGAAGATGTAA
AACAGGCTCCAAAACCTTCTCAGGCATCTAATGAAGCCCCAAAATCAAGTTCTCAA
TCTACAGAAGCTAATTCTCAGCAACAAGTTACTGCGAGTGAAGAGGCGGCTGTAG
AACAAGCAGTTGTAACAGAAAATACCCCTGCTACCAGTCAGGCACAACAAACTTA
TGCTGTTACTGAGACAACTTACAAACCTGCTCAACACCAGACAAGTGGCCAAGTAT
TGAGCAATGGAAATACTGCAGGGGCGGTCGGATCTGCTGCTGCAGCACAAATGGC
TGCTGCAACAGGAGTCCCTCAGTCTACTTGGGAACATATTATTGCCCGTGAATCAA
ATGGTAATCCTAATGTTGCTAATGCCTCAGGGAGCTTCAGGACTTTTCCAAACGAT
GCCAGGTTGGGGTTCAACAGCTACAGTTCAGGATCAAGTTAA

MNKRRKLSKLNVKKQHLAYGAITLVALFSCILAVTVIFKSSQVTTESLSKADKVRVAK
KSKMTKATSKSKVEDVKQAPKPSQASNEAPKSSSQSTEANSQQQVTASEEAAVEQAV
VTENTPATSQAQQTYAVTETTYKPAQHQTSGQVLSNGNTAGAVGSAAAAQMAAATG
VPQSTWEHIIARESNGNPNVANASGASGLFQTMPGWGSTATVQDQVNSAIKAYRAQG
LSAWGY*

ID-15

Clone 32

ATGATTGTTGGACACGGAATTGATTTACAAGAGATAGAGGCGATTACTAAAGCAT
ATGAGCGTAATCAACGTTTTGCAGAACGCGTTTTGACCGAACAAGAATTGCTTCTT
TTTAAAGGAATTTCCAATCCCAAGCGTCAGATGTCTTTTTTAACAGGGCGATGGGC
AGCAAAAGAGGCTTATAGCAAAGCACTTGGAACAGGAATTGGGAAAGTTAATTTT
CATGATATCGAAATTTTATCGGATGATAAAGGAGCGCCTTTGATTACAAAAGAACC

FIG. 1CONT'D

GTTTAATGGAAAATCTTTTGTTTCAATATCTCATAGTGGTAATTATGCACAAGCTAG
TGTTATTTTGGAGGAAGAAAAATGA

MIVGHGIDLQEIEAITKAYERNQRFAERVLTEQELLLFKGISNPKRQMSFLTGRWAAKE
AYSKALGTGIGKVNFHDIEILSDDKGAPLITKEPFNGKSFVSISHSGNYAQASVILEEEK*

ID-16

Clone 35

ATGATTTTTGTCACAGTGGGGACACATGAACAGCAGTTCAACCGTCTTATTAAAGA
AGTTGATAGATTAAAAGGGACAGGTGCTATTGATCAAGAAGTGTTCATTCAAACG
GGTTACTCAGACTTCGAACCTCAGAATTGTCAGTGGTCAAAATTTCTCTCATATGAT
GATATGAACTCTTACATGAAAGAAGCTGAGATTGTTATCACACATGGCGGCCCAGC
GACGTTTATGTCAGTTATTTCTTTAGGGAAATTACCAGTTGTTGTTCCTAGGAGAAA
GCAGTTTGGTGAACATATCAATGATCATCAAATACAATTTTTAAAAAAAATTGCCC
ACCTGTATCCCTTGGCTTGGATTGAAGATGTAGATGGACTTGCGGAAGCGTTGAAA
AGGAATATAGCTACAGAAAAATATCAGGGAAATAATGATATGTTTTGTCATAAATT
AGAAAAAATTATAGGTGAAATATGA

MIFVTVGTHEQQFNRLIKEVDRLKGTGAIDQEVFIQTGYSDFEPQNCQWSKFLSYDDM
NSYMKEAEIVITHGGPATFMSVISLGKLPVVVPRRKQFGEHINDHQIQFLKKIAHLYPL
AWIEDVDGLAEALKRNIATEKYQGNNDMFCHKLEKIIGEI*

ID-17

Clone39

TTGGAAGACAAATTATTCAACAAACATTTTATAGGCATTACTATTTTAAACTTTATT
GTTTATATGGTCTATTATTTGTTCACCGTTATCATAGCTTTTATTGCGACTAAAGAG
TTAGGTGTTAGCACTAGCCAAGCAGGATTAGCAACGGGGATTTATATTGTAGGGAC
TTTGATTGCTCGTCTTATATTTGGTAAGCAATTAGAAGTTCTAGGACGTAAGTTAGT
TTTACGTGGAGGGGCTATTTTTTACTTACTAACAACTTTAGCTTATTTTTATATGCC
AAGTATCGGAGTAATGTATTTAGTTCGTTTCCTAAATGGTTTTGGTTATGGCGTCGT
GTCAACAGCAACTAATACTATTGTAACAGCCTATATACCAGCTGATAAAAGAGGTG
AGGGGATTAACTTTTACGGTCTATCAACAAGTTTAGCCGCAGCTATTGGTCCTTTTG
TAGGAACATTTATGCTAGACAACCTTCATATTAACTTTAAAATGGTTATTGTATTAT
GTAGTATTTTAATTGCGATTGTAGTGTTGGGAGCATTTGTTTTCCCAGTCAAAAATA
TTACTTTAAATCCAGAACAGTTAGCTAAATCAAAATCATGGACTATTGATAGTTTC
ATTGAGAAAAAAGCAATTTTTATCACAATTATTGCATTTTTGATGGGTATCTCCTAT
GCTTCCGTGTTAGGTTTCCAAAAATTATATACAACAGAAATTAATTTGATGACAGT
AGGAGCTTATTTCTTTATTGTTTATGCACTTGTCATCACTTTAACCAGACCATCTAT
GGGAAGATTAATGGACGCTAAGGGAGATAAGTGGGTGCTTTATCCAAGTTATCTGT
TCTTAACTTTGGGACTTGCTTTATTAGGGAGTGCTATGGGAAGTGTTACCTACCTTC

FIG. 1CONT'D

TATCAGGTGCTTTGATTGGTTTTGGTTATGGCACCTTTATGTCTTGTGGCCAAGCAG
CATCAATCAAAGGTGTTGAGGAACATCGTTTCAATACAGCCATGTCAACTTACATG
ATAGGTCTTGATTTAGGGTTAGGTGCTGGACCTTACATTTTGGGACTTGTTAAAGAT
GGTTTTCTTGGAGCTGGTGTGCAATCCTTTAGAGAATTATTCTGGATAGCAGCGATT
ATTCCTGTTGTTTGTGGTATTCTATATTTCTTAAAATCATCTAGACAAGTTGAAACT
AAAACTATA
TAA

MEDKLFNKHFIGITILNFIVYMVYYLFTVIIAFIATKELGVSTSQAGLATGIYIVGTLIARL
IFGKQLEVLGRKLVLRGGAIFYLLTTLAYFYMPSIGVMYLVRFLNGFGYGVVSTATNTI
VTAYIPADKRGEGINFYGLSTSLAAAIGPFVGTFMLDNLHINFKMVIVLCSILIAIVVLG
AFVFPVKNITLNPEQLAKSKSWTIDSFIEKKAIFITIIAFLMGISYASVLGFQKLYTTEINL
MTVGAYFFIVYALVITLTRPSMGRLMDAKGDKWVLYPSYLFLTLGLALLGSAMGSVT
YLLSGALIGFGYGTFMSCGQAASIKGVEEHRFNTAMSTYMIGLDLGLGAGPYILGLVK
DGFLGAGVQSFRELFWIAAIIPVVCGILYFLKSSRQVETKTIZ

ID-18

Clone 47

ATGAATAGTGAACCTAAAAGTCAGTCAAACGAAGTAAAAAATAGCAAGCAATCAG
AAGTGAAGAAAGATAAAAAAATGACAAAAAAAGAACAATTAGCCTATCTCAAAG
AGCATGAGCAAGAAATCATAGATTATGTAAAATTACATAACAACCAAATTGAGTC
CGTTCAATTCGATTGGTCAAGTGTAAAAGTAGAACAAAGCGGGAATGGAACTCCA
CAAGGGGGTGATTATAATCTTTCACTGAGAGGAAAGTTTAATCATCTACAAAATTC
AAAATTAATAGTTGATTTTTATTTAGCTCATAAAAATGATATCCCAAATATCAAAT
CAATGGGAATGCTAAATAAGCCATATATACATAAAAATGGTATTTGGCACATTTAT
GAATAG

MILGGCQMNSEPKSQSNEVKNSKQSEVKKDKKMTKKEQLAYLKEHEQEIIDYVKLHN
NQIESVQFDWSSVKVEQSGNGTPQGGDYNLSLRGKFNHLQNSKLIVDFYLAHKNDIPN
IKSMGMLNKPYIHKNGIWHIYEZ

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Clone 102

ATGAAAAAGATTCGATTATCAAAGTTTATTAAAATGATTGTTGTTATTTTGTTTTTA
ATTAGTGTAGCAGCTAGTTTTTATTTTTTCCACGTTGCCCAAGTTCGAGATGATAAA
TCCTTTATTTCAAATGGTCAACGTAAGCCTGGAAACTCTTTATATGCTTATGATAAA
TCCTTTGATAAGCTATTAAAGCAAAAAATAGAAATGACAAACCAAAATATAAAGC
AAGTTGCTTGGTATGTTCCTGCTGCTAAGAAAACTCATAAGACAGTTGTTGTCGTTC
ATGGTTTTGCGAATAGCAAAGAGAATATGAAGGCATATGGTTGGCTGTTTCATAAG
TTAGGATACAATGTTCTTATGCCTGACAACATTGCACATGGTGAAAGTCATGGGCA

FIG. 1CONT'D

GTTGATAGGCTATGGCTGGAACGACCGCGAGAACATTATCAAATGGACAGAAATG
ATAGTGGATAAGAATCCATCAAGCCAAATTACTTTATTTGGTGTTTCAATGGGTGG
AGCAACAGTCATGATGGCTAGTGGTGAAAAATTACCTAGTCAGGTTGTTAATAT
CATTGAAGATTGTGGTTATTCTAGTGTTTGGGATGAATTAAAATTTCAGGCTAAAG
AGATGTATGGTTTACCAGCCTTCCCACTCTTATATGAAGTTTCAACAATTTCTAAAA
TCAGAGCAGGTTTTTCGTATGGACAAGCAAGTAGTGTCGAACAATTGAAAAAGAA
TAATTTACCAGCCCTCTTTATTCATGGTGATAAGGATAATTTTGTTCCAACAAGTAT
GGTTTATGACAACTATAAAGCTACAGCAGGTAAGAAAGAGCTTTATATTGTAAAA
GGGGCAAAACATGCGAAATCTTTTGAAACAGAGCCAGAAAAATATGAGAAACGTA
TCTCTAGTTTTTTGAAAAAATATGAAAAATAA

MKKIRLSKFIKMIVVILFLISVAASFYFFHVAQVRDDKSFISNGQRKPGNSLYAYDKSFD
KLLKQKIEMTNQNIKQVAWYVPAAKKTHKTVVVVHGFANSKENMKAYGWLFHKLG
YNVLMPDNIAHGESHGQLIGYGWNDRENIIKWTEMIVDKNPSSQITLFGVSMGGATV
MMASGEKLPSQVVNIIEDCGYSSVWDELKFQAKEMYGLPAFPLLYEVSTISKIRAGFSY
GQASSVEQLKKNNLPALFIHGDKDNFVPTSMVYDNYKATAGKKELYIVKGAKHAKSF
ETEPEKYEKRISSFLKKYEK*

ID-20

Clone 120

TTGAGGAGTAATATGGTAAAGACAGCAGTTTTAATGGCGACATACAATGGCGAAA
AATTTATATCTGAACAACTTGATTCAATTCGCCAACAGACATTAAAACCAGATTAT
GTATTATTGAGGGATGATTGTTCAACGGATGAAACAGTCAATGTCGTCAATAACTA
TATCGCAAAACATGAGTTAGAAGGCTGGAAAATTGTTAAAAACGACAAAAACTTA
GGCTGGCGTTTAAATTTTCGTCAATTACTTATTGATGTGTTAGCCTATGAGGTTGAC
TATGTCTTTTTTAGTGATCAAGATGATATTTGGTATCTTGATAAAAACGAACGACA
GTTTGCCATTATGTCAGATAACCCTCAAATTGAGGTTTTGAGTGCAGACGTTGATA
TCAAAACGATGTCTACAGAAGCCAGTGTTCCACATTTTCTAACTTTTTCTTCTAGTG
ATAGAATCAGTCAGTATCCTAAAGTATATGATTATCAAACATTCCGTCCCGGATGG
ACCATTGCTATGAAGAGAGATTTTGCGCAAGCTATCGCTTGA

MRSNMVKTAVLMATYNGEKFISEQLDSIRQQTLKPDYVLLRDDCSTDETVNVVNNYI
AKHELEGWKIVKNDKNLGWRLNFRQLLIDVLAYEVDYVFFSDQDDIWYLDKNERQF
AIMSDNPQIEVLSADVDIKTMSTEASVPHFLTFSSSDRISQYPKVYDYQTFRPGWTIAM
KRDFAQAIAZ

ID-21

Clone 143

ATGATTCATGAGATTCACGATTGTCAATTTATTGAAAAAGGAAGTTACGTTTATTT
GAATTATATTAATGCTGAGGGCGAGAGAGTAGTTATTATAATCATAGATTTTGTCC

FIG. 1CONT'D

GTAGTGTTAGTCCTATTTTATATCGTCTATTTATGATTTTACTTGCACAAGAAGTAC
CTCACTTGCATGATTACATCTATAATGCAAGAGATGATCACTACGATACTTGGAAG
TTTAAAGAATTAAAGGAGTCAAACCATCCAGTCCTTTTGGCATTCTCTGAAAGGTG
GCACGATAGTCGCTTGACTTCTAAAAGCCTTGCAGAATGTTTACAATTAACCGACC
TTGATGAAGAAGTGAAATCGACCATCATTCAATTAAGACAGTTCGAAAAATCAGTC
AGAAATCCTTTGGCTCACCTGATTAAACCTTTTGATGAGCAAGAACTATATCGTAC
AACTCAATTTTCTTCTCAAGCATTTTTAGACCAGATTATCTTCTTGGCAAAGGTAAT
TGGTGTTGAGTATGATACTGTTAATTTTCACTACGATACGGTTAACAAGCTTATTAT
AAAGATACTTGAGTAA

MIHEIHDCQFIEKGSYVYLNYINAEGERVVIIIIDFVRSVSPILYRLFMILLAQEVPHLHD
YIYNARDDHYDTWKFKELKESNHPVLLAFSERWHDSRLTSKSLAECLQLTDLDEEVKS
TIIQLRQFEKSVRNPLAHLIKPFDEQELYRTTQFSSQAFLDQIIFLAKVIGVEYDTVNFHY
DTVNKLIIKILE*

ID-22

Clone 1

ATGGTAAAAGTTTCAAATTTAGGGTATCCACGTCTTGGTGAACAGCGCGAATGGAA
GCAAGCGATCGAAGCTTTCTGGGCAGGGAATCTTGAACAAAAAGATTTAGAAAAA
CAACTAAAACAATTACGTATCAATCATTTAAAGAAACAAAAAGAGGCAGGTATTG
ACCTTATTCCAGTGGGGGATTTTTCTTGTTATGATCATGTTTTGGATTTGTCATTTCA
ATTCAATGTAATCCCAAAGCGTTTCGATGAGTATGAGAGGAATTTAGACCTTTATT
TTGCTATTGCAAGAGGTGACAAAGATAATGTCGCATCATCTATGAAAAAGTGGTTT
AATACCAACTACCACTACATAGTCCCAGAATGGGAGGTTGAGACTAAACCTCACTT
GCAGAATAATTACTTACTTGATCTTTATCTAGAAGCTAGGGAAGTAGTTGGTGATA
AAGCAAAGCCGGTTATC

MEEIMVKVSNLGYPRLGEQREWKQAIEAFWAGNLEQKDLEKQLKQLRINHLKKQKE
AGIDLIPVGDFSCYDHVLDLSFQFNVIPKRFDEYERNLDLYFAIARGDKDNVASSMKK
WFNTNYHYIVPEWEVETKPHLQNNYLLDLYLEAREVVGDKAKPVI

ID-23

Clone 2

ATGGTGTTACTTTTATTGCTAATGGTAGCCAAGTCAAGTTTGATGGTTACATGGCTG
TTTATAACGATACTGACAAAAATAAAATGTTACCAGATATGGAGGAAGGAGAAAG
TTATCAAGTTAA

MVLLLLLMVAKSSLMVTWLFITILTKIKCYQIWRKEKVIKL

ID-24

FIG. 1CONT'D

Clone 14

ATGAACAAAAAAATTTCCGGGATCGGCTTGGCTTCGATTGCAGTACT
TAGTTTAGCTGCATGTGGACATCGTGGTGCTTCTAAATCTGGTGGTAA
ATCAGATAGCTTGAAGGTTGCAATGGTAACAGATACCGGTGGTGTTG
ATGATAAATCATTTAACCAATCTGGTTGGGAAGGTATGCAAGCTTGG
GGCAAGAAGAATGGCCTTAAAAAAGGAGCTGGTTTTGACTATTTCCA
ATCGGCAAGTGAATCTGATTATGCAACTAACTTAGATACAGCTGTGT
CTAGTGGTTATAAATTGATTTTCGGTATTGGATTTTCTCTTCATGATG
CTATTGATAAAGCAGCAGACAATAACAAAGATGTTAATTACGTCATC
GTTGATGATGTTATTAAAGGGAAAGATAATGTTGCAAGTGTTGTCTTT
GCGGATAATGAATCAGCTTACTTAGCAGGTATTGCAGCCGCTAAAAC
TACCAAAACAAAAACAGTTGGCTTTGTAGGTGGTATGGAATCTGAGG
TTATTACCCGTTTTGAAAAAGGTTTTGAAGCAGGTGTCAAATCAGTTG
ATAAATCAATTAAAATTAAAGTTGACTATGCTGGTTCATTCGGTGAT
GCTGCTAAGGGTAAGACAATTGCAGCCGCACAATATGCTTCTGGCGC
AGATATT

MNKKISGIGLASIAVLSLAACGHRGASKSGGKSDSLKVAMVTDTGGVD
DKSFNQSGWEGMQAWGKKNGLKKGAGFDYFQSASESDYATNLDTAVS
SGYKLIFGIGFSLHDAIDKAADNNKDVNYVIVDDVIKGKDNVASVVFAD
NESAYLAGIAAAKTTKTKTVGFVGGMESEVITRFEKGFEAGVKSVDKSI
KIKVDYAGSFGDAAKGKTIAAAQYASGADI

ID-25

Clone 20

ATGTTACATTCTAAAAAAATACATTCCTTATCGCTTATTGCCGTTCTC
TCTTTAGCAACATATACGAGTTTACAACCAAATCATGTAGCGGCTGA
ACAATCACAAAAAACATCAACTGTTCTTATGAGTCAAAAAACTATTG
AACATAAGTTAAAAGTTGCAGATAAAGAAGCTGCTCCTCTCTACGCT
AAAATCGACCATATCCAACGACATATTGAAGTCAAAAAAGCAAAAG
ATTTAAAAGTTATTGAATTGTATATTAACAAAGATATCAACCAACTA
GAGAAGCAAAATAAACGTCTACTAACTAAATTCTATACTTCTATTGA
TAATCAAACATGGGATAGCACAAGTGAAGTCAAAAAATTGATTGATA
AGACAACCCTATCCACTAACGAAAAAGATAGATTAAAATTATATTTT
GAACAACGTGCTTACCTTGAGACAAGGTTGAACGACCGCTATCAAAA
ATTTGATAACTCTATTGAAAACCAAAATAAAGAACTAAAAATATTAA
CGTCAAAAATAGAAAAAATCTATCAAAAACATGGTATTACAAAAGA
GGTATTAAAAACTTACTATGCTAAAAAAACAGTACGAGCTGACTGA

FIG. 1CONT'D

MLHSKKIHSLSLIAVLSLATYTSLQPNHVAAEQSQKTSTVLMSQKTIEHK
LKVADKEAAPLYAKIDHIQRHIEVKKAKDLKVIELYINKDINQLEKQNK
RLLTKFYTSIDNQTWDSTSEVKKLIDKTTLSTNEKDRLKLYFEQRAYLET
RLNDRYQKFDNSIENQNKELKILTSKIEKIYQKHGITKEVLKTYYAKKTV
RAD*

ID-26

Clone 25

Clone 25 (partial sequence)

CTGAATTCCCAAAAACGCTACAATCAAACTTGGTATCCTACTTATGGTTTTTCTGAT
ACTTATGCATTCATGGTTACTAAAGAGTTTGCCAGACAGAATAAAATCACCAAGAT
CTCTGATCTCAAAAAGTTATCAACAACTATGAAGGCAGGGGTTGATAGTTCATGGA
TGAATCGCGAGGGAGATGGATACACTGATTTCGCTAAAACATACGGTTTTGAATTT
TCACATATTTACCCTATGCAAATTGGCTTAGTCTATGATGCGGTTGAAAGTAACAA
AATGCAATCTGTATTAGGCTACTCCACTGACGGTCGTATTTCGAGCTATGATTTAG
AAATTTTAAGGGATGATAAAAAATTCTTTCCTCCTTATGAAGCCTCTATGGTTGTCA
ACAATTCTATCATCAAAAAAGATCCTAAACTAAAAAAATTACTCCATCGACTCGAT
GGTAAAATCAATTTAAAAACGATGCAAAACCTTAATTATATGGTAGATGATAAACT
TTTAGAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCG
CTCACAATTCCACACAACATACGAGCCGGAAGCATAA

LNSQKRYNQTWYPTYGFSDTYAFMVTKEFARQNKITKISDLKKLSTTMKAGVDSSWM
NREGDGYTDFAKTYGFEFSHIYPMQIGLVYDAVESNKMQSVLGYSTDGRISSYDLEILR
DDKKFFPPYEASMVVNNSIIKKDPKLKKLLHRLDGKINLKTMQNLNYMVDDKLLEAW
RNHGHSCFLCEIVIRSQFHTTYEPEA*

ID-29

Clone 37

ATGAAAAAATTACTTTCCCTAACATGTCTAATCATGATGTCTTTATGT
TTAGTGGCATGTACTAAGCAAGCAATGTCGTCTAAGCAAGCAATGTC
GTCTAAGCAAATTAAAGATAAGAATAGTAAAGAAAAGGTGATTACT
GTTGCAACTTACAGCAAACCTACATCTACCTTTTTAGATTTGATTAAA
GATAATGTAAAAGAAAAAGGATATACTTTAAAGGTTGTCATGGTCTC
TGACTATATTCAGGCTAACATTGCTTTAGAAAACAAAGAACATGATG
CTAACCTTTTACAACATGAATTTTTCATGAGTATCTTTAATAAGGAAA

FIG. 1CONT'D

ATGATGGTCATCTAGTGTCAATTACACCAATTTATCATTCATTGGCTG
GTTTTTATGGTCAACATTTGAAAAATATTGCCGAGCTTAAAGACGGT
GCTAAGGTAGCGATTCCGTCTGATCCTGCCAATATGACTAGAGCTCT
GCTATTATTGCAAGAAAAGAAACTTATCACCTTAAAGAATACGTCCA
AAAAGACCAAGGCTATCGAAGATATTATTACTAACCCTAAAAAATTA
CGAATTGAACCTGTAGCATTACTTAACCTCAATCAGGCCTATTTTGAA
TATGACCTTGTCTTTAATTTCCCTGGATATGTGACAAAAATCAATCTA
GTTCCTAAAAGGGATAGATTATTATATGAGAAAAAACCAGATATCCG
TTTTGCAGGTGCCTTGGTAGCTCGTGAAGATAATAAAAATAGTGATA
AAATAAAAGTACTTAAAGAAGTACTAACAAGTAAAGAGATTCGTCA
CTATATCACTAAGGAGATTCCAAGTGAAGCAGACGTTGCGTTCTAG

MKKLLSLTCLIMMSLCLVACTKQAMSSKQAMSSKQIKDKNSKEKVITV
ATYSKPTSTFLDLIKDNVKEKGYTLKVVMVSDYIQANIALENKEHDANL
LQHEFFMSIFNKENDGHLVSITPIYHSLAGFYGQHLKNIAELKDGAKVAI
PSDPANMTRALLLLQEKKLITLKNTSKKTKAIEDIITNPKKLRIEPVALLN
LNQAYFEYDLVFNFPGYVTKINLVPKRDRLLYEKKPDIRFAGALVARED
NKNSDKIKVLKEVLTSKEIRHYITKEIPSEADVAF*

ID-30

Clone 38

CTGTTGGCTAAGGAAACCACTATGTCTGTCCTTTGGTATCAAAATTCTGCAGAAGC
CAAGGCTTTATATTTACAAGGTTATAATGTTGCTAAAATGAAGTTAGATGATTGGT
TACAAAAGCCCAGTGAAAAACCATATTCAATTATCTTAGATTTAGATGAAACAGTT
TTAGATAATAGCCCATATCAAGCAAAGAATATTAAAGATGGCTCTAGTTTCACGCC
AGAGAGTTGGGATAAATGGGTGCAAAAGAAATCAGCTAAGGCTGTTGCGGGTGCC
AAAGAATTTTTGAAGTATGCTAATGAAAAGGGAATAAAAATTTATTATGTCTCAGA
TCGTACAGATGCTCAAGTTGATGCGACTAAAGAAAATTTAGAGAAGGAAGGTATA
CCTGTTCAAGGGAAAGACCACTTGCTTTTCCTTAAAAAAGGAATGAAATCTAAAGA
GAGTCGCCGTCAGGCAGTTCAAAAAGATACCAATTTAATTATGCTTTTTGGAGATA
ATTTAGTTGATTTTGCTGATTTTTCTAAATCATCTAGTACAGATAGAGAACAACTAC
TAACTAAACTTCAAAGTGAGTTTGGTAGTAAATTTATTGTTTTCCCAAATCCTATGT
ACGGTTCTTGGGAAAGTGCTATTTATCAAGGAAAACATCTGGATGTTCAAAAACAA
TTGAAAGAACGACAAAAAATGTTGCATTCGTATGATTAA

MAKLTVKDVDLKGKKVLVRVDFNVPLKDGVITNDNRITAALPTIKYIIEQGGRAILFSH
LGRVKEEADKEGKSLAPVAADLAAKLGQDVVFPGVTRGAKLEEAINALEDGQVLLVE
NTRFEDVDGKKESKNDEELGKYWASLGDGIFVNDAFGTAHRAHASNVGISSNVEKAV
AGFLLENEIAYIQEAVETPERPFVAILGGSKVSDKIGVIENLLEKADKVLIGGGMTYTFY
KAQGIEIGTYLEKEDKLDVAKDSZ

ID-31

FIG. 1CONT'D

Clone 41

ATGGATAATAAAGGTAATAACGCCAATGTGATTGATGCAATCGCTGAGGGTGCAA
GCACAGGTGCACAAATGGCTTTCTCAATTGGTGCTAGTTTGATTGCCTTTGTTGGTT
TAGTTTCTTTGATTAA

MDNKGNNANVIDAIAEGASTGAQMAFSIGASLIAFVGLVSLI

ID-32

Clone 42

ATGAAAAAGAAAAACAAATCCTCTAACATTGCTATAATTGCAATCTT
TTTTGCTATTATGCTTGTCATTCATTTTTTGTCATCATTTATTTTTAGTT
TTTGGTTAGTCCCTATTAAACCTACTTTGATGCATATCCCAGTTATTA
TTGCATCTATAGCCTATGGACCTCGTATTGGTGCAACTCTAGGCGCCT
TAATGGGGGGGATCAGCGTAGCTAACAGCAGCATTGTTCTATTACCA
ACGAGTTACCTCTTCTCACCTTTTGTTGAAAATGGTAATTTTTATTCG
CTAATTATTGCACTTGTACCACGTATTCTAATCGGGATTATTCCTTAT
TTCGTTTACAAATTACTACACAACCGCTTTGGTTTGGCTATCTCAGGT
GCTATAGGCTCTCTAACAAACACAGTATTTGTTTTATCTGGAATTTTT
ATCTTTTTTTCAAGTACTTATAATGGGAATATCAAGCTAATGCTCGCT
GGGATTATTTCATCTAATTCATTAGCTGAGATGGTCATTGCAGCTATC
ATTGTATATCTAACTGATCCTCGTATTCTCAATATTAAACATTAA

MKKKNKSSNIAIIAIFFAIMLVIHFLSSFIFSFWLVPIKPTLMHIPVIIASIAY
GPRIGATLGALMGGISVANSSIVLLPTSYLFSPFVENGNFYSLIIALVPRILI
GIIPYFVYKLLHNRFGLAISGAIGSLTNTVFVLSGIFIFFSSTYNGNIKLML
AGIISSNSLAEMVIAAIIVYLTDPRILNIKH*

ID-33

Clone 43

TTGAATATGACATTACAAGACGAAATCAAAAAACGCCGTACTTTTGCCATCATCTC
TCACCCGGATGCTGGTAAGACGACTATTACTGAGCAATTATTATATTTTGGTGGTG
AAATTAGAGAAGCAGGGACAGTAAAAGGGAAAAAATCAGGTACTTTTGCAAAGTC
CGACTGGATGGATATTGAAAAGCAACGGGGTATCTCTGTTACTTCATCTGTTATGC
AATTTGATTACGCGGGTAAACGTGTTAA

MNMTLQDEIKKRRTFAIISHPDAGKTTITEQLLYFGGEIREAGTVKGKKSGTFAKSDW
MDIEKQRGISVTSSVMQFDYAG

FIG. 1CONT'D

KRV

ID-34

Clone 44

ATGGCAGATAAAAACAGAACATTTAAACTTGTAGGTGCAGGATCTTC
TAGCACACAAGAAAAAATTGAAAAGCCTGCTCTTTCGTTTATGCAAG
ATGCGTGGCGTCGCTTGAAAAAAAACAAATTAGCAGTAGTTTCACTC
TATTTATTAGCTCTTTTACTTACTTTTTCGTTAGCCTCAAATTTATTTG
TAACTCAGAAGGATGCTAATGGGTTTGATTCGAAAAAAGTAACGACA
TATCGCAACTTACCACCTAAATTGAGTTCAAACCTTCCTTTTTGGAAT
GGTAGCATTAATCCATCA

MADKNRTFKLVGAGSSSTQEKIEKPALSFMQDAWRRLKKNKLAVVSLY
LLALLLTFSLASNLFVTQKDANGFDSKKVTTYRNLPPKLSSNLPFWNGSI
NPS

ID-35

Clone 46

ATGAAAAGAAAACAGTTTATAAAATTAGGAATTGCAACCTTACTAACGGTTATTTC
GCTTTACACACCAATAAACCTAGCTACAAATCATACCACAGAAAATATTGTTACTG
CTCAAGAGTATAAAACAAAGAGAATGGTACTTTACCTTTTAA

MKRKQFIKLGIATLLTVISLYTPINLATNHTTENIVTAQEYKTKENILFLL

ID-36

Clone 50

ATGTTTTATAATCCTTTACTTTTTATTGTACTAATTACAATTGCTGTATTTTTCTTAG
CTAAGAAAAAATGGCAATTACCGACATTTACTTTCATTGGTTTGCTATTTATCTATA
ACCAAGGGCTGTGGGAACAGTTGATTAAT

MFYNPLLFIVLITIAVFFLAKKKWQLPTFTFIGLLFIYNQGLWEQLIN

FIG. 1 CONT'D

ID-37

Clone 51/52

GTGGTGCAAATAATGAAAAAACATATAAAAAGTATCATACCAATAGT
TCTTATTGGTATGATACTAGGAGGCTGTCAAATGAATAGTGAACATA
AAAGTCAGTATAATGAAACAAAAAGTAGCAAGCAATCAGAAGTGAA
GAAAGATAAAAAAATGACAAAAAAAGAACAATTAGCTTATCTCAAA
GAGCATGAACAAGAAATAATTGATTTTGTAAAATCTCAGAATAAAAA
GATAGAATCTGTACAAATTGATTGGAATGATGTTCGATGGAGTAAAG
GGGGAAATGGTACACCTCAAGGAGGAGGAGAGGGGATTTTACTTTTT
GGGGAGATTAATAATGATTCTGAATCAAGTTGGAGAGTTGATATTGA
TATAGAAAAAGGACGGCTAGACCTAAAAAATATGTATTTAGGACAA
CCTATACGAATTGGAGGTAAATTATTTGAGTAA

MVQIMKKHIKSIIPIVLIGMILGGCQMNSEHKSQYNETKSSKQSEVKKDK
KMTKKEQLAYLKEHEQEIIDFVKSQNKKIESVQIDWNDVRWSKGGNGT
PQGGGEGILLFGEINNDSESSWRVDIDIEKGRLDLKNMYLGQPIRIGGKLF
E*

ID-38

Clone 53

ATGGAATTTTTGGCTTATAATGCTTTCACAGCAATCGGTGTTTCTATT
CCGCACGGTAATCATTTCCACTTTATTCACTATAAGGATATGTCTCCA
TTAGAGTTAGAAGCAACAAGGATGGTGGCAGAGCATAGAGGACATC
ATATTGATGCATTAGGGAAAAAAGATTCTACAGAGAAACCAAAGCA
TATTTCTCATGAACCTAATAAGGAACCTCACACAGAGGAAGAACACC
ATGCAGTAACACCGAAAGACCAACGTAAAGGCAAACCAAATAGCCA
GATTGTCTACAGTGCTCAAGAAATTGAAGAGGCAAAAAAAGCTGGT
AAATACACAACATCTGATGGTTACATTTTTGATGCTAAAGATATTAA
AAAAGATACAGGTACAGGTTATGTCATTCCACATATGACACATGAGC
ATTGGGTACCAAAGAAAGATTTATCAGAGTCGGAATTAAAAGCAGCT
CAAGAATTTCTTTCAGGAAAATCTGAAGCAAATCAAGACAAACCAAA
AACAGGTAAAACAGCTCAAGAAATCTATGAGGCAATTGAACCAAAA
GCAATTGTTAAACCTGAAGATTTATTATTTGGAATTGCACAAGCGAC
AGACTATAAGAATGGTACATTTGTAATTCCTCATAAAGATCATTACC
ATTATGTGGAATTAAAATGGTTTGATGAAGAAAAAGATCTTTTAGCT
GATTCAGATAAGACATATTCTTTAGAAGACTATTTAGCTACGGCTAA
ATATTACATGATGCACCCAGAAAAACGTCCTAAAGTTGAAGGATGGG
GTAAAGATGCTGAAATTTATAAGGAAAAGGACTCTAATAAAGCAGA
TAAACCAAGTCCTGCACCAACTGATAATAAATCAACATCAAATTCTA

FIG. 1CONT'D

GTGACAAAAACTTAAGTGCTGCAGAAGTATTCAAACAAGCAAAACC
AGAAAAAATTGTACCGCTTGATAAAATTGCTGCTCACATGGCATATG
CAGTTGGATTTGAAGATGATCAATTGATTGTTCCTCATCATGATCATT
ATCATAATGTTCCTATGGCATGGTTTGACAAGGGTGGTTTATGGAAA
GCACCAGAAGGCTATACATTACAACAACTCTTCTCAACAATTAAATA
CTACATGGAACATCCTAATGAATTACCAAAAGAAAAGGGTTGGGGA
CACGACAGTGATCATAACAAAGGCTCAAATAAAGACAATAAAGCCA
AAAATTATGCTCCAGATGAAGAACCTGAAGATTCAGGGAAAGTAACT
CACAACTATGGTTTTTATGATGTTAATAAAGGTTCAGACGAAGAAGA
ACCAGAAAAACAAGAAGATGAATCAGAGCTAGATGAATATGAACTA
GGAATGGCACAAAACGCTAAGAAATATGGTATGGATAGACAATCTTT
TGAAAAGCAACTCATCCAATTATCAAATAAATATAGTGTAAGTTTTG
AAAGC

MEFLAYNAFTAIGVSIPHGNHFHFIHYKDMSPLELEATRMVAEHRGHHI
DALGKKDSTEKPKHISHEPNKEPHTEEEHHAVTPKDQRKGKPNSQIVYS
AQEIEEAKKAGKYTTSDGYIFDAKDIKKDTGTGYVIPHMTHEHWVPKK
DLSESELKAAQEFLSGKSEANQDKPKTGKTAQEIYEAIEPKAIVKPEDLL
FGIAQATDYKNGTFVIPHKDHYHYVELKWFDEEKDLLADSDKTYSLED
YLATAKYYMMHPEKRPKVEGWGKDAEIYKEKDSNKADKPSPAPTDNK
STSNSSDKNLSAAEVFKQAKPEKIVPLDKIAAHMAYAVGFEDDQLIVPH
HDHYHNVPMAWFDKGGLWKAPEGYTLQQLFSTIKYYMEHPNELPKEK
GWGHDSDHNKGSNKDNKAKNYAPDEEPEDSGKVTHNYGFYDVNKGS
DEEEPEKQEDESELDEYELGMAQNAKKYGMDRQSFEKQLIQLSNKYSV
SFES

ID-39 (Same as ID-76)

Clone 56

ATGAGGAAACGTTTTTCCTTGCTAAATTTTATTGTTGTTACTTTTATTT
TCTTTTTCTTTATTCTTTTTCCGCTTTTTAAGGCCAAAGATTGTCAGGT
TGTTTATGCAAGTTTTCAAGGAGATCATTGGGACATTTGTAACGCATT
TGATTTTCCGTATTTACATCGCTTTGATCTCATTAAAGGTAAAGAAAA
TCAACTTTACTTTATAGGTTGTACAATTGCTAACAGTAAAGCCTACAC
TGAGGATTGGAGTGATAAAGGCCGAATTTTTGTTGCTCGTTTTAATAC
TCAAAACCATACATTGGAAGGATTGCAACAATTGCCTCAAACTTTAT
TAAAAAATCATGGATACTATGCCATTCAGGATGAAGGATATTCATTG
ATTACTTCAGTAGAAGGGGTACTCAAACTCACTTATCCAGAATTTTCT
ACTACAGGCGACTGGCAATTAGAACGGCTTTTCGATGAGGAGACAAG
CGATGTGGTGAAAGTGGATATTAATCAGGATGGTAAGGATGAGTATG
TGATCATCCAAGGTTTTCATGGAGATCGTTTACGTATCTTCACTGAAG
ATTTCGGTCGAGAATTATTCCATTATCCTGAAAAAACCCCATTTGGTC
ACGCTATTTGGAGTGGTCGTTTACTTAATCAGACTTGTTTCGTATTCG

FIG. 1 CONT'D

GGTGGCGATCAGAAAAAGCAGAATTAAGGCTTTTTCACTTTGTAGAT
GGGCACTTGGTTTCAGAATTAGTAGATGCAAAAGCAGCTTCTAGTAA
TGTCTTAGCTTTTGAAAAAGATGGAAAAGCTTATCTTTTCTCAGCCAA
TAACGGACGTGGCGAAGTTGCTCTTTATCAATTAGTAAAATAA

MRKRFSLLNFIVVTFIFFFFILFPLFKAKDCQVVYASFQGDHWDICNAFDF
PYLHRFDLIKGKENQLYFIGCTIANSKAYTEDWSDKGRIFVARFNTQNHT
LEGLQQLPQTLLKNHGYYAIQDEGYSLITSVEGVLKLTYPEFSTTGDWQ
LERLFDEETSDVVKVDINQDGKDEYVIIQGFHGDRLRIFTEDFGRELFHY
PEKTPFGHAIWSGRLLNQTCFVFGWRSEKAELRLFHFVDGHLVSELVDA
KAASSNVLAFEKDGKAYLFSANNGRGEVALYQLVK*
ID-40

Clone 57

ATGAAGCACAAGTTAAAAGCTTTTACGCTTGCTTTACTCTCAATATTC
TTTGTGTTTGGTGGAAAGGTCAGTGCAGAGACTGTGAATATTGTTTCT
GATACAGCATACGCTCCATTCGAATTTAAAGATTCTGATCAAACTTAT
AAAGGAATCGATGTTGACATCGTTAACGAAGTCGCTAAGCGTGCTGG
CTGGAATGTTAACATGACGTATCCAGGTTTTGATGCCGCAGTTAACG
CTGTTCAATCTGGACAGGCAGATGCGCTAATGGCCGGAACTACTGTT
ACTGAAGCACGTAAAAAAGTCTTTAATTTCTCAGATACTTATTACGAT
ACTTCCGTTATTCTTTATACTAAAAATAATAATAAAGTCACAAACTAC
AAACAACTAAAAGGAAAAGTAGTCGGTGTAAAAAATGGAACAGCTG
CTCAAAGCTTCTTAGAAGAAAATAAATCTAAATACGGCTATAAAGTT
AAAACATTTGATACAAGCGACCTAATGAATAACAGCCTTGATTCTGG
TTCTATTTACGCCGCTATGGACGATCAACCAGTTGTGCAATTTGCGAT
AAATCAAGGAAAAGCTTACGCCATTAACATGGAAGGCGAAGCAGTT
GGTAGCTTTGCATTTGCTGTCAAAAAAGGTAGTGGACACGATAATCT
AATTAAAGAATTTAACACAGCTTTTGCACAAATGAAATCAGATGGCA
CTTATAATGACATCATGGATAAATGGCTTGGAAAAGACGCTACAAAA
ACAAGCGGCAAAGCAACAGGTAATGCCAATGAAAAAGCAACTCCTG
TAAAGCCAAGTTATAAAATTGTTTCTGATTCTTCATTCGCACCATTCG
AATATCAAAACGGTAAAGGGAAATATACTGGTTTTGATATGGAATTA
ATCACGAAAATTGCTAAACAGCAAGGTTTTAAACTTGATATCTCAAA
TCCAGGTTTTGATGCCGCTTTAAATGCTGTCCAATCTGGGCAAGCTGA
CGGTGTTATTGCAGGAGCCACAATCACAGAAGCACGCCAAAAAATCT
TTGATTTTTCTGATCCTTATTACACATCTAGCGTTATCTTAGCGGTTAA
AAAAGGAAGCAATGTCAAATCATACCAAGATTTAAAAGGAAAAACA
GTTGGTGCTAAAAATGGTACTGCCTCATATACTTGGTTATCAGACCAC
GCAGATAAGTACAACTATCATGTTAAAGCATTTGATGAAGCATCTAC
AATGTATGATAGTATGAACTCAGGTTCAATTGATGCTCTAATGGATG
ACGAAGCCGTTCTTGCTTACGCTATTAATCAAGGTCGTAAATTTGAA
ACACCTATCAAAGGTGAAAAATCAGGCGATATCGGATTTGCAGTGAA

FIG. 1CONT'D

AAAAGGGGCAAATCCAGAATTAATTAAAATGTTTAACAACGGTCTTG
CTTCACTCAAAAAATCGGGTGAGTACGATAAACTTGTTAAAAAATAC
CTTTCCACAGCCAGCACTTCTTCAAACGATAAAGCTGCTAAACCTGT
AGATGAATCAACTATTTTAGGGTTAATTTCTAATAACTACAAACAATT
GCTATCTGGTATTGGAACTACTTTAAGTTTAACTCTTATCTCGTTTGC
GATTGCTATGGTTATTGGTATTATCTTTGGTATGATGAGCGTATCACC
AAGTAATACTCTCCGCACAATTTCAATGATTTTTGTTGATATTGTCCG
TGGTATTCCACTCATGATTGTGGCCGCTTTTATTTTCTGGGGTATTCCT
AATTTAATCGAAAGCATCACAGGTCACCAAAGTCCAATTAATGACTT
CGTTGCTGCTACTATCGCTCTTTCTTTAAATGGTGGTGCGTACATTGC
TGAAATTGTACGTGGTGGTATTGAAGCTGTTCCTTCTGGTCAAATGGA
AGCAAGTCGCAGCTTAGGTATTTCTTACGGCAAAACTATGCAAAAGG
TTATCTTACCTCAAGCAGTACGCCTTATGTTACCAAACTTTATCAACC
AATTTGTCATCTCATTAAAGGATACAACAATTGTATCAGCAATCGGA
CTTGTGGAACTCTTCCAAACTGGTAAATCATAA

MKHKLKAFTLALLSIFFVFGGKVSAETVNIVSDTAYAPFEFKDSDQTYK
GIDVDIVNEVAKRAGWNVNMTYPGFDAAVNAVQSGQADALMAGTTV
TEARKKVFNFSDTYYDTSVILYTKNNNKVTNYKQLKGKVVGVKNGTA
AQSFLEENKSKYGYKVKTFDTSDLMNNSLDSGSIYAAMDDQPVVQFAI
NQGKAYAINMEGEAVGSFAFAVKKGSGHDNLIKEFNTAFAQMKSDGTY
NDIMDKWLGKDATKTSGKATGNANEKATPVKPSYKIVSDSSFAPFEYQ
NGKGKYTGFDMELITKIAKQQGFKLDISNPGFDAALNAVQSGQADGVIA
GATITEARQKIFDFSDPYYTSSVILAVKKGSNVKSYQDLKGKTVGAKNG
TASYTWLSDHADKYNYHVKAFDEASTMYDSMNSGSIDALMDDEAVLA
YAINQGRKFETPIKGEKSGDIGFAVKKGANPELIKMFNNGLASLKKSGEY
DKLVKKYLSTASTSSNDKAAKPVDESTILGLISNNYKQLLSGIGTTLSLTL
ISFAIAMVIGIIFGMMSVSPSNTLRTISMIFVDIVRGIPLMIVAAFIFWGIPN
LIESITGHQSPINDFVAATIALSLNGGAYIAEIVRGGIEAVPSGQMEASRSL
GISYGKTMQKVILPQAVRLMLPNFINQFVISLKDTTIVSAIGLVELFQTGK
S*

ID-41

Clone 58

TTGGAAGGTTTACTTATTGCATTGATTCCCATGTTTGCGTGGGGAAGTATTGGATTT
GTTAGTAATAAAATTGGAGGGCGTCCAAATCAACAAACATTTGGAATGACTTTAGG
AGCATTGCTATTTGCGATTATCGTATGTTTATTTAA

MEGLLIALIPMFAWGSIGFVSNKIGGRPNQQTFGMTLGALLFAIIVCLF

FIG. 1CONT'D

ID-42

Clone 70

ATGAATACTATTTATAATACATTGAGAACAGATAAAGGTTATAAAGT
TTATGAGGGGTATTTATATGAAATTACTGGTGAAGAATGTGAAGAAG
CCTTAGACCTTGTGATTCCTAAGAATATTGTATTTGCAGATACAGATA
CTTGTGGCTACACTTTTTTACTCAATGAAGATGGAACAGTTTATGATG
ATGTGACTTTCTACAAATTTGATGATAAATATTGGTTGGCTAGTCATA
AAGCTTTGGATTCTTATTTAGACAACATCAATTTTGACTATACCGTAA
CAGATATTTCTGACGAGTATAAAATGCTGCAAATTGAAGGAAGATAT
TCGGGAGAAATTGCTCAGTCATTTTATGAATATGATATTTCAACACTT
AATTTTCGTACTCTTCGCATAGAGATGGACTTCATCAAAGGTGAGGA
AAGGTTATCTTGGCGTAGATTTGGTTTTTCTGGAGAATTTGGCTATCA
ATTTTTCCTACCATCTTCTATTTTTGCTACTTTTGTTTCGGATGTCTGT
GAAGGTATAGCAGAGTGTGGGGATGAACTTGATAGATATTTAAGGTT
TGAAGTGGGACAACCCATTACTGATATTTATCAACAAGAAGAATATT
CTTTATATGAAATAGGTTATTCTTGGAATCTAGATTTCACAAAGGAA
GAATTTAGAGGTCGCGATAGCTTGTTAGAGCACATCAGATCAGCAAC
AGTTAAAAGTGTTGGATTCTCAACGAAGGAAAAACTCGCTTCAGGAA
CACCAGTGCTATTTGATGACCAAATTGTTGGAAAGATTTTTTGGATAG
CAGACGAGAAACACTCTTCGGAAAATTACCTAGGTTTGATGATTGTT
AACCAAACATATGCTCATTCAGGAGTTACTTTTGTAACAGAAGATGG
CCAAATTTTGAAAACACAATCAAGCCCTTATTGTATCCCAGAAAGTT
GGAACAAAGAATGA

MNTIYNTLRTDKGYKVYEGYLYEITGEECEEALDLVIPKNIVFADTDTCG
YTFLLNEDGTVYDDVTFYKFDDKYWLASHKALDSYLDNINFDYTVTDIS
DEYKMLQIEGRYSGEIAQSFYEYDISTLNFRTLRIEMDFIKGEERLSWRRF
GFSGEFGYQFFLPSSIFATFVSDVCEGIAECGDELDRYLRFEVGQPITDIY
QQEEYSLYEIGYSWNLDFTKEEFRGRDSLLEHIRSATVKSVGFSTKEKLA
SGTPVLFDDQIVGKIFWIADEKHSSENYLGLMIVNQTYAHSGVTFVTED
GQILKTQSSPYCIPESWNKE*

ID-43

Clone 78/94

ATGGAGTTAGTAATTAGAGATATTCGTAAGCGGTTTCAGGAAACAGA
GGTCTTGAGAGGAGCAAGTTACCGATTTTATTCAGGTAAAATAACAG
GGGTCTTAGGTAGGAATGGTGCTGGGAAAACAACTTTATTTAATATA
CTTTATGGGGATCTTGCAGCTGACAACGGGACCATTTGTTTATTGAAG
GATAATCACGAGTATCCTCTTACCGATAAGGATATTGGTATTGTTTAT

FIG. 1CONT'D

TCCGAAAACTACCTTCCAGAATTTTTAACAGGGTATGAATTTGTAAA
ATTTTACATGGATTTACATCCTTCAGATGATTTAATGACAATAGATGA
TTATTTAGATTTTATGGAAATAGGACAAACAGAGCGTCATAGAATTA
TCAAAGGATATTCTGATGGAATGAAGAGTAAGCTCTCATTAATTTGC
CTGATGATTTCTAAGCCAAAAGTAATTTTACTAGATGAGCCACTGAC
TGCAGTTGATGTTGTATCAAGTATTGCAATAAAACGCCTTTTGTTGGA
ATTAAGTGAGGATCATATTATTATATTATCAACTCATATAATGGCCTT
AGCAGAAGATCTATGTGATATTGTGGCTGTATTAGACAAAGGAAAAC
TCCAAACATTAGATATTGATCGTAAACATGAACAATTCGAAGAGCGT
CTTCTTCAAGTGTTGAAGGGAGATGAATATGACAAGTAA

MELVIRDIRKRFQETEVLRGASYRFYSGKITGVLGRNGAGKTTLFNILYG
DLAADNGTICLLKDNHEYPLTDKDIGIVYSENYLPEFLTGYEFVKFYMD
LHPSDDLMTIDDYLDFMEIGQTERHRIIKGYSDGMKSKLSLICLMISKPK
VILLDEPLTAVDVVSSIAIKRLLLELSEDHIIILSTHIMALAEDLCDIVAVL
DKGKLQTLDIDRKHEQFEERLLQVLKGDEYDK*

ID-44

Clone 80

TTGTTTATGAGATATACAAATGGAAATTTTGAAGCCTTTGCAAGACCT
CGAAAACCTGAAGGTGTGGATAAAAAATCCGCTTATATTGTTGGTTC
TGGTTTAGCAGGATTAGCTGCCGCTGTCTTTTTAATACGTGACGGTCA
AATGGATGGTCAACGTATTCATATTTTTGAAGAACTACCTCTTTCTGG
AGGATCACTTGACGGTGTCAAACGACCTGATATCGGTTTTGTAACGC
GTGGTGGTCGTGAAATGGAAAATCACTTCGAATGTATGTGGGATATG
TACCGTTCCATCCCCTCTCTCGAAGTTCCAGATGCTTCTTATCTAGAT
GAATTTTATTGGCTTGACAAGGATGATCCCAATTCATCTAACTGTCGC
CTCATTCATAAACAGGGGAATCGCTTAGAATCTGATGGTGATTTTAC
ACTCGGAACACATTCCAAAGAGTTAGTTAAGCTAGTCATGGAGACTG
AAGAGTCTTTAGGTGCTAAGACGATTGAAGAAGTTTTTTCAAAAGAA
TTTTTTGAAAGTAATTTTTGGACTTATTGGGCTACTATGTTTGCCTTTG
AGAAATGGCATTCAGCGATTGAAATGCGTCGATATGCTATGCGCTTT
ATCCATCATATTGGTGGTCTGCCTGATTTCACTTCATTAAAATTTAAT
AAATATAATCAATATGATTCTATGGTGAAACCAATCATCAGTTATTTA
GAGTCTCATAATGTAGATGTTCAATTTGATAGCAAGGTAACTAATAT
CTCCGTAGACTTT

MFMRYTNGNFEAFARPRKPEGVDKKSAYIVGSGLAGLAAAVFLIRDGQ
MDGQRIHIFEELPLSGGSLDGVKRPDIGFVTRGGREMENHFECMWDMY
RSIPSLEVPDASYLDEFYWLDKDDPNSSNCRLIHKQGNRLESDGDFTLGT
HSKELVKLVMETEESLGAKTIEEVFSKEFFESNFWTYWATMFAFEKWHS

FIG. 1CONT'D

AIEMRRYAMRFIHHIGGLPDFTSLKFNKYNQYDSMVKPIISYLESHNVDV
QFDSKVTNISVDF

ID-45

Clone 81

TTGTTGGCTTCTTTATTTATCGTCCGTTTGTCAAAATCGCTTTCGCTAA
GGAGGAGCAATATGAAAAAATTACTTAGATGGCTTCCTCCTGTACTT
TTCATTATTATCCTTATAGGAATGACTATCTTAGGTAAGTCCTATATC
AATAAAGTAACAGCTCACAAAATAAAACTCTATAACTCTCGAATGAC
TCCTACTATTTTAATTTCAGGATCCAGTGCTACTCAAGAACGATTTAA
CAGCATGTTAGCACAGCTCAACCAAATGGGAGAAAAACATAGCGTTT
TAAAGTTAACTGTCAAAAAAGACAATAGCATTATCTACAATGGACAA
ATTAGCGGCAATGACCACAAACCCTACATTGTCATTGGATTTGAAAA
TAATGAAGATGGTTATAGTAACATCAAAAAACAAACAAAATGGCTA
CAGATTGCTATGAATGATCTTCAGAAGAAATATAAATTTAAACGTTT
TAACGCTATCGGTCATTCAAATGGTGGCTTATCATGGACTATTTTCCT
AGAAGATTATTACGACTCTGATGAATTTGATATGAAATCATTGTTAA
CAATGGGAACACCTTTTAACTTTGAAGAAAGTAACACCTCAAATCAT
ACTCAAATGCTTAAAGATTTAATCAGTAATAAAGGAAATATTCCATC
AAGTCTCATGGTATACAATTTGGCAGGAACTAATTCATATGATGGTG
ATAAAATTGTTCCATTTGCTAGTGTGGAGACTGGTAAATATATTTTCC
AAGAAACCGCTAAACACTATACCCAACTAACAGTAACTGGTAATAAT
GCTACACATTCTGACTTGCCTGATAATCCTGAAGTTATCCAATATGTC
GCAGAAAAAATTCTTAAAAATGAGAAAGGTAAATTACCAAAACCTC
ACTAA

MLASLFIVRLSKSLSLRRSNMKKLLRWLPPVLFIIILIGMTILGKSYINKVT
AHKIKLYNSRMTPTILISGSSATQERFNSMLAQLNQMGEKHSVLKLTVK
KDNSIIYNGQISGNDHKPYIVIGFENNEDGYSNIKKQTKWLQIAMNDLQK
KYKFKRFNAIGHSNGGLSWTIFLEDYYDSDEFDMKSLLTMGTPFNFEES
NTSNHTQMLKDLISNKGNIPSSLMVYNLAGTNSYDGDKIVPFASVETGK
YIFQETAKHYTQLTVTGNNATHSDLPDNPEVIQYVAEKILKNEKGKLPK
PH
*

ID-46

Clone 83

TTGAAATTAGGTATTACAACATTCGGAGAGACAACAATCCTTGAAGAAACAAACC
AAAGCTATTCACATCCTGAGAGGATTCGCCAATTAGTTGCTGAGATTGAACTAGCT
GATCAAGTTGGTTTAGATGTATATGGTATTGGAGAGCACCATCGTGAAGATTTTGC

FIG. 1 CONT'D

GGTCTCTGCACCCGAAATTATCCTAGCAGCAGGAGCGGTTAGAACTAATAATATCC
GTTTATCTAGTGCAGTAACGATTCTCTCTTCCAATGATCCTATTCGCGTCTATCAGC
AATTTTCAACGATTGACGCACTTTCAAATGGTAGAGCAGAAATTATGGCAGGGCGT
GGTTCCTTTATTGAGTCTTTTCCATTGTTTGGATACGATTTAGCGGATTATGATGAT
TTATTTAATGAAAAAATGGATATGTTGTTAGCAATTAACTCAGCGACAAATCTCGA
TTGGAAAGGTCATTTGACACAAACAGTTAATGAGCGACCAATTTATCCAAGAGCAT
TACAAAGACAGTTATCAATATGGGTGGCAACAGGAGGAAATGTTGATTCTACAATT
CGTATTGCAGAACAAGGTTTGCCAATTGTTTATGCAACTATTGGTGGGAATCCCAA
AGCCTTTCGTCAATTGGTCCATATTTATAAAGAAGTTGGTAAGTCCGTAATGGACA
CAAACCAGGAACAACTAAAAGTTGCTGCTCACTCTTGGGGATGGATTGAAGAGGA
TAATCAAACCGCTATTGACCGTTATTTTTTCCCTACGAAACAGACCGTCGATAATAT
TGCTAAGGGACGCCCTCATTGGTCTGAAATGACTAAAGAGCAGTATTTACGTTCAA
TAGGTCCAGAAGGTGCTATTTTTGTAGGAAATCCTGAAGTGGTTGCACATAAAATT
ATAGGACTTTGGTGA

MKLGITTFGETTILEETNQSYSHPERIRQLVAEIELADQVGLDVYGIGEHHREDFAVSAP
EIILAAGAVRTNNIRLSSAVTILSSNDPIRVYQQFSTIDALSNGRAEIMAGRGSFIESFPLF
GYDLADYDDLFNEKMDMLLAINSATNLDWKGHLTQTVNERPIYPRALQRQLSIWVAT
GGNVDSTIRIAEQGLPIVYATIGGNPKAFRQLVHIYKEVGKSVMDTNQEQLKVAAHSW
GWIEEDNQTAIDRYFFPTKQTVDNIAKGRPHWSEMTKEQYLRSIGPEGAIFVGNPEVV
AHKIIGLW

ID-47

Clone 86

ATGATAGAGTGGATTCAAACACATTTACCAAATGTATATCAAATGGG
TTGGGAAGGTGCTTACGGCTGGCAGACAGCTATTGTACAAACCCTTT
ATATGACTTTTTGGTCGTTCCTTATTGGAGGTTTAATGGGATTGTTAG
GAGGTTTATTCCTTGTTTTAACTAGTCCTAGAGGAGTTATTGCTAATA
AATTAGTATTTGGAGTTTTAGATAAAGTTGTTTCTGTTTTTAGAGCTC
TGCCCTTCATTATTCTTCTTGCTTTGATTGCGCCAGTAACTCGCGTAAT
TGTAGGAACAACACTTGGTTCACCAGCAGCTTTGGTACCTCTTTCTTT
GGCAGTTTTCCCATTTTTTGCTCGTCAAGTTCAAGTTGTTTTAGCTGA
ACTTGATGGTGGAGTTATTGAGGCTGCACAAGCCTCAGGTGGAACAC
TTTGGGATATTATTGTAGTTTATCTTCGTGAAGGTCTACCAGATTTAA
TTCGAGTATCAACGGTTACTTTGATTTCTTTAGTAGGTGAAACAGCTA
TGGCTGGCGCTATTGGTGCAGGAGGATTGGGTTCTGTTGCTATTACTA
AAGGATATAACTATTCTCGTGATGATATTACTTTAGTAGCGACTATTC
TGATTTTATTATTAATTTCTTTATCCAATTTTTAGGTGATTTTTTAAC
ACGTCGCTTGAGTCATAAATAA

MIEWIQTHLPNVYQMGWEGAYGWQTAIVQTLYMTFWSFLIGGLMGLL
GGLFLVLTSPRGVIANKLVFGVLDKVVSVFRALPFIILLALIAPVTRVIVG

FIG. 1CONT'D

TTLGSPAALVPLSLAVFPFFARQVQVVLAELDGGVIEAAQASGGTLWDII
VVYLREGLPDLIRVSTVTLISLVGETAMAGAIGAGGLGSVAITKGYNYSR
DDITLVATILILLLIFFIQFLGDFLTRRLSHK*

ID-48 (same as ID-43)

ID-49

Clone 96

TTGGCAGTTAGTTTTCATGAAGTATTTGGTTGGGATTCTGCTTTTTTA
TTATGATTATCAATATTCCATTGCTCCTTCTTTGCTACTTTGGCTTAGG
TAAACAAACCTTTTTAAAAACTGTCTATGGTTCTTGGATTTTTCCTGT
TTTTATTAAGTTAACACAAAGTGTACCAACTTTGACCCACAACTCACT
CCTCGCAGCACTTTTTGGAGGTGTTATTGTAGGATGTGGTTTGGGGAT
TGTTTTTTGGAGCGACTCTTCAACTGGTGGAACGGGGATTATCATTCA
ATTCTTAGGAAAATATACTCCTATAAGCCTTGGACAAGGGGTTATAT
TGATTGATGGACTTGTTACAATTGTTGGTTTCCTAGCTTTTGACAGTG
ATACGGTTATGTTTTCTATTATTGGGTTGATAACTATTAGTTATATTAT
TAATGCTATCCAAACTGGATTTACAACCTTAAGCACTGTCTTAATCGT
TTCTCAAGAGCACCAAAAAATTAAGACATATATCAATACTGTCGCAG
ATAGAGGAGTAACAGAAATTCCCGTTAAAGGGGGATATTCTGGAACT
AATCAAATCATGCTTATGACAACTATTGCTGGTTATGAGTTTGCTAAA
TTACAAGAGGCAATAGCAGAAATTGACGAAACAGCCTTCATAACAGT
AACTCCAACATCACAAGCTTCTGGACGTGGATTTAGTCTTCAAAAAA
ATCATGGACGTCTTGATGAAGACATTCTTATGCCAATGTAA

MAVSFHEVFGWDSAFFIMIINIPLLLLCYFGLGKQTFLKTVYGSWIFPVFI
KLTQSVPTLTHNSLLAALFGGVIVGCGLGIVFWSDSSTGGTGIIIQFLGKY
TPISLGQGVILIDGLVTIVGFLAFDSDTVMFSIIGLITISYIINAIQTGFTTLST
VLIVSQEHQKIKTYINTVADRGVTEIPVKGGYSGTNQIMLMTTIAGYEFA
KLQEAIAEIDETAFITVTPTSQASGRGFSLQKNHGRLDEDILMPM*

ID-50

Clone 99

ATGAAAGAAAAACAGTCGAAAAGGCTTATTTATATACTACTGATTGTTCCCATTAT
CTTTATAAGTGTTTTTACATACAGTATTAGCCAGCCTTCTAAACTACTTCCACCAAA
AGAATTAGTTATTCTAAGTCCAAATAGTCAAGCCATTTTAACAGGAACGATTCCAG
CTTTTGAGGAAAAATACGGTATAAAAGTTAAGCTTATTCAAGGTGGGACAGGGCA
ACTAATAGATAGATTAAGTAAGGAGGGTAAGCAGTTGAAGGCGGATATTTTCTTTG
GAGGAAATTATACGCAATTTGAAAGTCATAAGGCATTGTTTGAGTCTTACGTATCA

FIG. 1CONT'D

AAGAATGTTCATACTGTTATTCCAGACTATATCCATCCGAGTGATACGGCGACACC
TTATACTATAAATGGGAGTGTCTTGATTGTAAATAACGAATTAGCTAAGGGACTTA
CCATCAAGAGTTATGAAGATTTATTACAGCCTTCCTTAAAAGGTAAAATTGCCTTT
GCAGATCCTCTAGAGTCGACCTGCAAGCATGCAAGCTTGGCGTAA

MKEKQSKRLIYILLIVPIIFISVFTYSISQPSKLLPPKELVILSPNSQAILTGTIPAFEEKYGI
KVKLIQGGTGQLIDRLSKEGKQLKADIFFGGNYTQFESHKALFESYVSKNVHTVIPDYI
HPSDTATPYTINGSVLIVNNELAKGLTIKSYEDLLQPSLKGKIAFADPLESTCKHASLA

ID-51

Clone 103

CCTCCTATCAAATGATGACAAACGTGAGAGGTACATGGAACAAATGCTCTTTAAAA
TTGAAAATGCAACCTGGCAGCGTGTGGTAAGAGCACTTTATCGTAAATACAATAAG
GAATTTTTTACATATCCAGCCGCCAAAACAAACCACCACGCTTTTGAATCAGGATT
GGCATATCACACGGCAACAATGGTTCGTTTGGCAGATAGTATCGGAGATATCTATC
CAGAACTTAATAAAAGTTTGATGTTTGCTGGTATTATGCTACATGATTTAGCCAAG
GTCATAGAGTTATCGGGTCCTGATAATACAGAATATACTATTCGAGGTAATCTTAT
CGGTCATATTTCACTTATTGATGAGGAATTAA

LLSNDDKRERYMEQMLFKIENATWQRVVRALYRKYNKEFFTYPAAKTNHHAFESGL
AYHTATMVRLADSIGDIYPELNKSLMFAGIMLHDLAKVIELSGPDNTEYTIRGNLIGHIS
LIDEEL

ID-52

Clone 104

ATGAAAAAAAATAAAATTATCCGATTCAGTTTAGTTGGTGTTCTACTT
GCGATACTATGCTTTAGTCTTTTTGCTTTATTGAAGCCTAACAGTCAA
CAATCATCATCTCAAAAGTTGAGGAATGAGGATATAAAAAAGACATC
CTCTCAAAAAAGAAATAAGAAATTACGATTACCAGCTGTATCATCAA
AAGATTGGAACTTGATTTTGGTCAATCGTGACCATAAACATGAAGAA
TTAAGTCCAGATGTGGTGCCTGTTGAAAATATTTATTTGGATAAACGT
ATTACGAAGCAAGCTACTCAGTTTTTAGAGGCTGCTAGAGCAATTGA
TTCACGAGAACATTTAATTTCGGGTTATCGTAGTGTTGCCTATCAGGA
GAAGTTGTTCAATTCTTATGTTACTCAAGAGATGACTAGTAACCCTAA
TTTGACGAGGGGACAAGCAGAAAAGTTGGTAAAAACTTACTCTCAGC
CTGCAGGTGCTAGTGAACACCAGACTGGATTAGCGATGGATATGAGT
ACTGTAGATTCTTTGAATGAGAGCGATCCTAGAGTAGTCAGTCAGTT
GAAAAAGATAGCTCCACAATATGGTTTTGTCTTACGGTTTCCGGATG
GTAAAACAGCAGAAACAGGGGTAGGTTATGAAGATTGGCATTACCG

FIG. 1 CONT'D

CTATGTTGGGGTAGAGTCTGCAAAATATATGGTCAAACATCATTTAA
CATTAGAAGAATACATAACTTTATTAAAGGAGAATAACCAATGA

MKKNKIIRFSLVGVLLAILCFSLFALLKPNSQQSSSQKLRNEDIKKTSSQK
RNKKLRLPAVSSKDWNLILVNRDHKHEELSPDVVPVENIYLDKRITKQA
TQFLEAARAIDSREHLISGYRSVAYQEKLFNSYVTQEMTSNPNLTRGQA
EKLVKTYSQPAGASEHQTGLAMDMSTVDSLNESDPRVVSQLKKIAPQY
GFVLRFPDGKTAETGVGYEDWHYRYVGVESAKYMVKHHLTLEEYITLL
KENNQ*

ID- 53

Clone 106

CTGTTATGTGGATTTCTTCCATCAATTCCTGTGTCTAATTCCGGGGGG
TATGGTATAATAACAGTTATGAAAAATAAAAAAATCTTATTTGGGAC
TGGCCTTGCTGGTGTGGGTTTACTGGCAGCTGCTGGTTATACCCTAAC
TAAAAAAGTAACAGATTATAAACGTCAGCAAATCACTCAGACCTTAA
GAGAACTTTTTAGTCAGATGGGTGATATTCAGGTATTTTATTTTAATG
AATTTGAATCTGATATTAAAATGACCAGTGGTGGTCTTGTCTTGGAA
GATGGCAGAATTTTCGAATTCATTTATCGTCAAGGTGTTCTTGATTAT
GTGGAGGTGAGCAAATGA

LLCGFLPSIPVSNSGGYGIITVMKNKKILFGTGLAGVGLLAAAGYTLTKK
VTDYKRQQITQTLRELFSQMGDIQVFYFNEFESDIKMTSGGLVLEDGRIF
EFIYRQGVLDYVEVSK*

ID-54

Clone 108

ATGTATCAAACTCAGACAAATAAGGAAAAATTTGTTTTATTTTTGAAATTATTTATC
CCAGTATTGATTTATCAATTTGCTAATTTTTCAGCTACTTTTATTGATTCGGTTATGA
CTGGACAGTATAGTCAGCTACATTTGGCAGGTGTGTCAACTGCTAGTAATTTATGG
ACTCCGTTTTTCGCTTTATTAGTAGGTATGATTTCAGCATTAGTACCAGTAGTTGGT
CAACATTTGGGTAGAGGAAATAAAGAACAAATTCGCACAGAATTTCATCAATTTCT
ATATTTAGGTTTGATACTGTCCTTAA

MYQTQTNKEKFVLFLKLFIPVLIYQFANFSATFIDSVMTGQYSQLHLAGVSTASNLWTP
FFALLVGMISALVPVVGQHLGRGNKEQIRTEFHQFLYLGLILSL

ID-55

FIG. 1CONT'D

Clone 112

CTGCTCTTTTTAGCTAACTTTTCTAATTTATGGTATAATTGTATGGATT
GTTTAGCTAGAATGGAGAAGATGATGCAAGATGTTTTCATTATAGGA
AGTAGAGGGTTGCCAGCTCGTTACGGTGGTTTTGAAACTTTTGTTTCA
GAATTGATTAATCATCAAAAAAGTTCCGACATAAAATACCATGTTGC
ATGCCTTAGTGATAAAGAACATCATACTCATTTTAACTTTGCTGACGC
TGATTGTTTTACTATAAATCCTCCCCAATTAGGGCCAGCACGTGTGAT
TGCTTATGATATTATGGCCATTAATTATGCCCTTGACTTGGTTAAGAC
ACATGATTTAAAAGAGCCTATTTTTTATATTTTAGGAAATACAATTGG
TGCCTTTATTTGGCATTTTGCCAATAAAATACATAAAGTCGGTGGCTT
ATTGTATGTTAATCCGGATGGTTTAGAGTGGAAGCGATCAAAGTGGT
CTCGTCCCACACAGCGTTATTTAAAATACGCCGAAAAATGTATGACT
AAAAATGCAGACCTAATTATTTCTGATAATATTGGTATTGAAAATTA
CATTCAATCTACCTACTCTAATGTGAAGACAAGGTTCATTGCTTACGG
TACAGAGATTAATTCTAGGAAATTATCGTCAGATGATCCACGTGTCA
AACAGTTGTTTAAAAAATGGAATATTAAGTCTAAGGGTTACTATCTA
ATCGTTGGTCGATTTGTCCCTGAAAACAATTATGAAACGGCTATTAG
GGAGTTCATGGCTTCAGATACTAAGCGTGATTTAGTTATTATCTGTAA
CCATCAAAATAACCCCTACTTTGAAAAGTTGTCCTTAAAGACAAACC
TTCAACAAGATAAAAGAGTTAAGTTTGTAGGTACGCTCTATGAAAAA
GATCTGCTGGATTATGTTCGTCAACAAGCCTTTGCTTATATTCATGGG
CATGAAGTTGGCGGTACTAATCCAGGACTGCTTGAGGCTTTAGCTAA
TACTGATTTGAATCTTGTTCTAGATGTTGATTTCAACAAATCAGTAGC
AGGTCTCTCAAGTTTTTACTGGACTAAAAAAGAGGGGGATTTAGCTA
AGCTT

MLFLANFSNLWYNCMDCLARMEKMMQDVFIIGSRGLPARYGGFETFVS
ELINHQKSSDIKYHVACLSDKEHHTHFNFADADCFTINPPQLGPARVIAY
DIMAINYALDLVKTHDLKEPIFYILGNTIGAFIWHFANKIHKVGGLLYVN
PDGLEWKRSKWSRPTQRYLKYAEKCMTKNADLIISDNIGIENYIQSTYSN
VKTRFIAYGTEINSRKLSSDDPRVKQLFKKWNIKSKGYYLIVGRFVPENN
YETAIREFMASDTKRDLVIICNHQNNPYFEKLSLKTNLQQDKRVKFVGT
LYEKDLLDYVRQQAFAYIHGHEVGGTNPGLLEALANTDLNLVLDVDFN
KSVAGLSSFYWTKKEGDLAKL

ID-56

Clone 120

TTGAGGAGTAATATGGTAAAGACAGCAGTTTTAATGGCGACATACAA
TGGCGAAAAATTTATATCTGAACAACTTGATTCAATTCGCCAACAGA
CATTAAAACCAGATTATGTATTATTGAGGGATGATTGTTCAACGGAT
GAAACAGTCAATGTCGTCAATAACTATATCGCAAAACATGAGTTAGA

FIG. 1CONT'D

AGGCTGGAAAATTGTTAAAAACGACAAAAACTTAGGCTGGCGTTTAA
ATTTTCGTCAATTACTTATTGATGTGTTAGCCTATGAGGTTGACTATG
TCTTTTTTAGTGATCAAGATGATATTTGGTATCTTGATAAAAACGAAC
GACAGTTTGCCATTATGTCAGATAACCCTCAAATTGAGGTTTTGAGTG
CAGACGTTGATATCAAAACGATGTCTACAGAAGCCAGTGTTCCACAT
TTTCTAACTTTTTCTTCTAGTGATAGAATCAGTCAGTATCCTAAAGTA
TATGATTATCAAACATTCCGTCCCGGATGGACCATTGCTATGAAGAG
AGATTTTGCGCAAGCTATCGCTTGA

MRSNMVKTAVLMATYNGEKFISEQLDSIRQQTLKPDYVLLRDDCSTDET
VNVVNNYIAKHELEGWKIVKNDKNLGWRLNFRQLLIDVLAYEVDYVFF
SDQDDIWYLDKNERQFAIMSDNPQIEVLSADVDIKTMSTEASVPHFLTFS
SSDRISQYPKVYDYQTFRPGWTIAMKRDFAQAIA*

ID-57

Clone 123

GTGATTATGGATAAGTCTATTCCTAAAGCAACTGCTAAACGTTTATCA
CTGTACTACCGTATTTTTAAACGTTTTAATACTGATGGCATCGAAAAA
GCTAGTTCCAAACAAATTGCAGATGCCCTAGGTATCGATTCTGCTACT
GTTCGACGTGATTTTTCTTATTTTGGTGAACTAGGACGCCGTGGTTTT
GGTTATGATGTCAAAAAACTTATGAACTTCTTTGCAGAAATATTGAA
CGATCATTCTACAACAAATGTTATGCTGGTGGGGTGTGGAAATATCG
GTAGAGCTCTCTTGCATTATCGTTTCCACGATCGCAATAAAATGCAA
ATTTCAATGGCTTTTGATTTAGATAGCAATGATTTAGTTGGTAAAACA
ACCGAGGATGGAATTCCTGTCTACGGTATTTCGACTATCAATGACCA
TTTAATAGATAGTGATATTGAAACTGCTATCCTAACAGTACCTAGTAC
AGAAGCCCAAGAAGTTGCTGACATCTTAGTCAAAGCAGGTATAAAA
GGCATCTTGAGTTTTTCTCCAGTTCATTTAACATTACCAAAAGATATC
ATTGTTCAGTATGTAGATTTAACAAGCGAATTACAAACTTTACTTTAT
TTCATGAACCAGCAGCGATAA

MIMDKSIPKATAKRLSLYYRIFKRFNTDGIEKASSKQIADALGIDSATVRR
DFSYFGELGRRGFGYDVKKLMNFFAEILNDHSTTNVMLVGCGNIGRALL
HYRFHDRNKMQISMAFDLDSNDLVGKTTEDGIPVYGISTINDHLIDSDIE
TAILTVPSTEAQEVADILVKAGIKGILSFSPVHLTLPKDIIVQYVDLTSELQ
TLLYFMNQQR*

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Clone 125

FIG. 1 CONT'D

ATGGGTGCTAAAGGAGCAGATGTCATTCTCGTTTTATCACACTCTGGCATTGGAGA
TGATCGATATGAAGAAGGTGAAGAAAACGTTGGCTATCAAATTGCCAGCATCAAG
GGAGTGGATGCCGTTGTTACGGGACACTCACACGCTGAATTTCCATCAGGTAACGG
TACTGGCTTCTATGAAAAATACACTGGAGTTGATGGTATCAATGGAAAAATAAATG
GAACACCTGTTACAATGGCAGGCAAGTACGGGGATCACCTTGGTATTATTGATTTA
GGACTTAGTTATACTAATGGAAAATGGCAAGTCTCCGAAAGCAGTGCTAAAATCC
GTAAAATTGATATGAACTCAACAACTGCTGACGAGCGTATCATTGCATTGGCTAAG
GAAGCACACGATGGCACTATCAACTATGTTCGCCAACAAGTAGGTACAACAACTG
CGCCAATTACAAGTTACTTTGCACTAGTTAA

MGAKGADVILVLSHSGIGDDRYEEGEENVGYQIASIKGVDAVVTGHSHAEFPSGNGTG
FYEKYTGVDGINGKINGTPVTMAGKYGDHLGIIDLGLSYTNGKWQVSESSAKIRKIDM
NSTTADERIIALAKEAHDGTINYVRQQVGTTTAPITSYFALV

ID-59

Clone 135

TTGTCAATAAGGTTTCAAATCAGCTTGAAATATGATAAAATAAAACAGATTGTAAG
TGACTGTTTAAGCTTGTTTTCAGAGAGGTTTTATGAATACAAACACAATAAAAA
AGGTTGTAGCGACTGGAATTGGAGCTGCACTTTTTATCATTATAGGTATGCTAGTT
AA

MSIRFQISLKYDKIKQIVSDCLSLFFREVFMNTNTIKKVVATGIGAALFIIIGMLV

ID-60

Clone 145

ATGAAACATTTAAAATTTCAATCGGTCTTCGACATTATTGGTCCTGTTATGATTGGA
CCATCAAGTAGTCATACTGCAGGAGCTGTCCGCATTGGTAAAGTTGTCCATTCTAT
TTTTGGTGAACCTAGTGAAGTAACCTTTCATTTATACAATTCTTTTGCTAAAACTTA
CCAAGGACACGGTACTGATAAAGCATTGGTTGCAGGGATTCTAGGAATGGATACA
GATAATCCAGATATTAA

MKHLKFQSVFDIIGPVMIGPSSSHTAGAVRIGKVVHSIFGEPSEVTFHLYNSFAKTYQG
HGTDKALVAGILGMDTDNPDI

ID-61

Clone 147

FIG. 1 CONT'D

GTGTCAGAAGGTGTTTTAATGTTTCTAAAAGAAGATGACGTAGAGACTTTTCTTCA
TATCCTGACAAATTCATTTAGCCAATTTATGGCACAATTTGATTTGTGTCATAAGGA
AATGATTAA

ID-62

Clone 150

ATGACCTACAAAGATTACACAGGTTTAGATCGGACTGAACTTTTGAGTAAAGTGCG
TCATATGATGTCCGACAAACGTTTTAA

MTYKDYTGLDRTELLSKVRHMMSDKRF

ID-63

Clone S2

CTGAGTTGGGTCTTGGAAACGGTCCTGTCAATCATACTAGCTATCAAGGAGACTAA
AATGTATTTAGAACAACTAAAAGAGGTAAATCCTTTAA

MSWVLETVLSIILAIKETKMYLEQLKEVNPL

ID-67

Clone 3-40

GTGAAAAAAAAATTAGTCTCATCACTTCTAAAGTGTTCTCTAATCATT
ATTGTTAGCTTTGCTGGTGGAGCATTTGCTAGTTTTGTCATGAATCAT
AATGACAATATTCCAAATGGTGGTGTCACTAAAACTAGTAAAGTAAA
TTATAATAACATAACGCCTACAACAAAAGCTGTTAAAAAGGTACAAA
ATAGTGTTGTTTCTGTTATCAATTATAAACAACAAGAGAGTCGTTCTG
ACCTATCAGACTTCTATAGTCATTTTTTTGGTAATCAGGGGGGCAACA
CTGATAAGGGCTTACAAGTTTACGGTGAAGGCTCTGGAGTCATCTAT
AAAAAAGATGGTAAAAATGCCTATGTTGTCACTAATAACCACGTCAT
TGATGGGGCTAAACAAATTGAAATTCAACTAGCTGATGGCTCAAAAG
CAGTTGGGAAACTTGTTGGGTCAGATACCTACTCTGATTTAGCCGTCG
TCAAAATTCCATCAGATAAAGTTTCAAATATTGCAGAATTTGCTGATT
CATCAAAACTCAACATTGGTGAAACTGCTATAGCGATCGGAAGCCCT
CTTGGAACTGAGTATGCAAATTCTGTAACTCAAGGTATTGTATCTAGT
TTAAAAAGAACTGTAACAATGACTAATGAAGAAGGACAAACAGTTT
CTACAAATGCTATCCAGACGGATGCTGCTATCAATCCTGGTAATTCA
GGTGGAGCACTTATCAATATTGAAGGACAGGTTATTGGAATTAATTC
TAGTAAAATTTCTTCTACATCAAATCAAACCTCAGGACAATCGTCAG

FIG. 1CONT'D

GAAATAGCGTTGAAGGTATGGGATTTGCCATTCCTTCAAATGATGTT
GTTAAGATTATCAATCAACTTGAGAGTAACGGACAAGTAGAGAGACC
TGCTCTAGGTATTTCTATGGCTGGATTAAGTAATTTACCATCCGATGT
TATTAGTAAACTGAAAATCCCAAGTAATGTTACTAATGGTATTGTAG
TAGCATCTATCCAATCTGGCATGCCAGCTCAAGGCAAACTAAAGAAA
TACGATGTCATTACTAAAGTTGACGATAAAGAAGTAGCATCTCCAAG
TGATTTACAAAGTTTACTCTATGGCCACCAGGTAGGGGATTCCATAA
CAGTAACCTTTTATCGTGGTGAAAATAAACAAACAGTCACTATAAAA
CTTACTAAAACTAGTAAAGATTTAGCTAAACAACGAGCAAATAACTA
A

MKKKLVSSLLKCSLIIIVSFAGGAFASFVMNHNDNIPNGGVTKTSKVNY
NNITPTTKAVKKVQNSVVSVINYKQQESRSDLSDFYSHFFGNQGGNTDK
GLQVYGEGSGVIYKKDGKNAYVVTNNHVIDGAKQIEIQLADGSKAVGK
LVGSDTYSDLAVVKIPSDKVSNIAEFADSSKLNIGETAIAIGSPLGTEYAN
SVTQGIVSSLKRTVTMTNEEGQTVSTNAIQTDAAINPGNSGGALINIEGQ
VIGINSSKISSTSNQTSGQSSGNSVEGMGFAIPSNDVVKIINQLESNGQVE
RPALGISMAGLSNLPSDVISKLKIPSNVTNGIVVASIQSGMPAQGKLKKY
DVITKVDDKEVASPSDLQSLLYGHQVGDSITVTFYRGENKQTVTIKLTKT
SKDLAKQRANN*

ID-68

Clone 3-30

ATGTTAAAATGGTATACAAACAAAGGAGGGAGGATGATAATGAAGA
AATGTTTTTTGGCTATTTGTTTAGCTCTTAGTTTTTTATGGTTTCAGT
TCAAGCAGATGAGGTGGACTATAACATTCCTCATTATGAGGGTAATC
TAACTATTCACAATGATAATAGTGCTGATTTTACAGAGAAGGTTACTT
ACCAATTTGATTCGTCCTATAATGGACAGTATGTCACGTTAGGTACG
GCGGGTAAGTTATCTGACAATTTTGATATTAATAATAAGCCACAGGT
TGAAGTTTCAATTAATGGTAAAGTAAGGAAAGTTAGTTACCAGATAG
AAGATTTGGAGGATGGCTACCGTTTGAAAGTGTTTAATGGTGGTGAA
GCAGGTGATACTGTTAAAGTCAATGTTCAGTGGAAACTAAAAAATGT
TCTATTTATGCATAAGGATGTTGGTGAACTTAACTGGATTCCTATTAG
CGACTGGGATAAAACGTTAGAGAAAGTAGATTTTTGGATATCAACTG
ACAAAAAGGTTGCTCTTTCTCGTCTTTGGGGGCACTTGGGTTATCTTA
AAACTCCTCCTAAAATAAGACAAAATAATAATCGTTACCATTTGACA
GCTTTTAATGTAAACAAACGATTAGAATTTCATGGTTATTGGGATAG
ATCTTATTTTAATCTACCTACAAACAGTAAAAATAATTACAAGAAAA
AAATTGAACATCAAGAGAAGATAATAGAGCGTCATGGTTTTATCCTA
AGTTTCTTGTTAAGGATATTATTACCTTCATTCTTTATTATTGTGACAC
TATTCATCTCAATTAGGGTGTTCCTGTTTAGAAAAAAAGTTAATAAAT

FIG. 1CONT'D

ACGGGCAATTCCCTAAGGATCATCATTTATATGAAGCACCTGAGGAC
CTTTCACCATTAGAGTTAACTCAAAGCATTTATAGTATGAGCTTTAAA
AATTTTCAAGATGAGGAGAAGAAAACTCACCTTATCAGTCAAGAACA
ACTCATACAGTCAATTCTATTAGACTTGATTGATAGAAAAGTATTGA
ATTATGATGATAACTTGTTATCTCTAGCTAACTTAGATAGAGCTTCTG
ATGCAGAAATAGATTTTATAGAGTTTGCTTTTGCGGATTCTACGAGTT
TGAAGCCAGATCAACTCTTTTCTAATTACCAATTTAGTTATAAAGAAA
CACTACGTGAACTGAAAAAGCAGCACAAGGCTTCAGATCTGCAAAAT
CAAATGAGACGCCGAGGAAGTAATGCCTTATCAAGAATTACGCGTCT
CACAAGGTTGATTTCTAAAGACAATATAAACTCTCTTAGAAGAAAGG
GAATTTCATCCCCTTATCGTAAAATGTCTTCAGAAGAGTCTAAAGAA
TTATCTAGGTTAAAAAGATTCAGTTACCTATCACCTCTTATTTCTTTTG
TTGTTATAATTTATACGCTTTTTTTAAATTATTTTACCTATTTCTGTAT
CTATCTCTTATTGTTTGGTGTTATCCTGTTGTTGAATAAAATCATTTTT
ATGATGACAAGAAAAATAAGTAACGGTTATATTGTAACTGAAGATGG
AGCAAGTCGTGTCTACCAATGGACTAGTTTTAGGAACATGCTAAGGG
ATATCAAATCGTTTGATCGTTCAGAGTTAGAAAGTATCGTATTATGG
AATCGAATATTGGTTTACGCTACTTTATTCGGCTACGCTGACCGTGTT
GAGAAAGTACTCAGAGTGAACCAAATAGATATTCCAGAAAGATTTGC
AAACATTGATAGTCATCGATTTGCGATTTCAGTCAATCAATCTAGTAA
TCATTTTTCAACGATAACTGAAGATGTTAGTCACGCTTCTAATTTTAG
TGTTAATTCAGGCGGTTCTTCAGGTGGTTTCTCAGGCGGCGGAGGCG
GCGGAGGTGGCGGTGCCTTCTAA

MLKWYTNKGGRMIMKKCFLAICLALSFFMVSVQADEVDYNIPHYEGNL
TIHNDNSADFTEKVTYQFDSSYNGQYVTLGTAGKLSDNFDINNKPQVEV
SINGKVRKVSYQIEDLEDGYRLKVFNGGEAGDTVKVNVQWKLKNVLF
MHKDVGELNWIPISDWDKTLEKVDFWISTDKKVALSRLWGHLGYLKTP
PKIRQNNNRYHLTAFNVNKRLEFHGYWDRSYFNLPTNSKNNYKKKIEH
QEKIIERHGFILSFLLRILLPSFFIIVTLFISIRVFLFRKKVNKYGQFPKDHHL
YEAPEDLSPLELTQSIYSMSFKNFQDEEKKTHLISQEQLIQSILLDLIDRKV
LNYDDNLLSLANLDRASDAEIDFIEFAFADSTSLKPDQLFSNYQFSYKET
LRELKKQHKASDLQNQMRRRGSNALSRITRLTRLISKDNINSLRRKGISS
PYRKMSSEESKELSRLKRFSYLSPLISFVVIIYTLFLNYFTYFCIYLLLFGVI
LLLNKIIFMMTRKISNGYIVTEDGASRVYQWTSFRNMLRDIKSFDRSELE
SIVLWNRILVYATLFGYADRVEKVLRVNQIDIPERFANIDSHRFAISVNQS
SNHFSTITEDVSHASNFSVNSGGSSGGFSGGGGGGGGGAF*

ID-69

Clone 3-38
ATGATGATTGTGAATAATGGTTATCTAGAAGGGAGAAAAATGAAAA
AGAGACAAAAAATATGGAGAGGGTTATCAGTTACTTTACTAATCCTG

FIG. 1CONT'D

TCCCAAATTCCATTTGGTATATTGGTACAAGGTGAAACCCAAGATAC
CAATCAAGCACTTGGAAAAGTAATTGTTAAAAAAACGGGAGACAAT
GCTACACCATTAGGCAAAGCGACTTTTGTGTTAAAAAATGACAATGA
TAAGTCAGAAACAAGTCACGAAACGGTAGAGGGTTCTGGAGAAGCA
ACCTTTGAAAACATAAAACCTGGAGACTACACATTAAGAGAAGAAA
CAGCACCAATTGGTTATAAAAAAACTGATAAAACCTGGAAAGTTAAA
GTTGCAGATAACGGAGCAACAATAATCGAGGGTATGGATGCAGATA
AAGCAGAGAAACGAAAAGAAGTTTTGAATGCCCAATATCCAAAATC
AGCTATTTATGAGGATACAAAAGAAAATTACCCATTAGTTAATGTAG
AGGGTTCCAAAGTTGGTGAACAATACAAAGCATTGAATCCAATAAAT
GGAAAAGATGGTCGAAGAGAGATTGCTGAAGGTTGGTTATCAAAAA
AAAATCCAGGGGTCAATGATCTCGATAAGAATAAATATAAAATTGAA
TTAACTGTTGAGGGTAAAACCACTGTTGAAACGAAAGAACTTAATCA
ACCACTAGATGTCGTTGTGCTATTAGATAATTCAAATAGTATGAATA
ATGAAAGAGCCAATAATTCTCAAAGAGCATTAAAAGCTGGGGAAGC
AGTTGAAAAGCTGATTGATAAAATTACATCAAATAAAGACAATAGA
GTAGCTCTTGTGACATATGCCTCAACCATTTTTGATGGTACTGAAGCG
ACCGTATCAAAGGGAGTTGCCGATCAAAATGGTAAAGCGCTGAATG
ATAGTGTATCATGGGATTATCATAAAACTACTTTTACAGCAACTACA
CATAATTACAGTTATTTAAATTTAACAAATGATGCTAACGAAGTTAA
TATTCTAAAGTCAAGAATTCCAAAGGAAGCGGAGCATATAAATGGG
GATCGCACGCTCTATCAATTTGGTGCGACATTTACTCAAAAAGCTCTA
ATGAAAGCAAATGAAATTTTAGAGACACAAAGTTCTAATGCTAGAAA
AAAACTTATTTTTCACGTAACTGATGGTGTCCCTACGATGTCTTATGC
CATAAATTTTAATCCTTATATATCAACATCTTACCAAAACCAGTTTAA
TTCTTTTTTAAATAAAATACCAGATAGAAGTGGTATTCTCCAAGAGG
ATTTTATAATCAATGGTGATGATTATCAAATAGTAAAAGGAGATGGA
GAGAGTTTTAAACTGTTTTCGGATAGAAAAGTTCCTGTTACTGGAGG
AACGACACAAGCAGCTTATCGAGTACCGCAAAATCAACTCTCTGTAA
TGAGTAATGAGGGATATGCAATTAATAGTGGATATATTTATCTCTATT
GGAGAGATTACAACTGGGTCTATCCATTTGATCCTAAGACAAAGAAA
GTTTCTGCAACGAAACAAATCAAAACTCATGGTGAGCCAACAACATT
ATACTTTAATGGAAATATAAGACCTAAAGGTTATGACATTTTTACTGT
TGGGATTGGTGTAAACGGAGATCCTGGTGCAACTCCTCTTGAAGCTG
AGAAATTTATGCAATCAATATCAAGTAAAACAGAAAATTATACTAAT
GTTGATGATACAAATAAAATTTATGATGAGCTAAATAAATACTTTAA
AACAATTGTTGAGGAAAAACATTCTATTGTTGATGGAAATGTGACTG
ATCCTATGGGAGAGATGATTGAATTCCAATTAAAAAATGGTCAAAGT
TTTACACATGATGATTACGTTTTGGTTGGAAATGATGGCAGTCAATTA
AAAAATGGTGTGGCTCTTGGTGGACCAAACAGTGATGGGGGAATTTT
AAAAGATGTTACAGTGACTTATGATAAGACATCTCAAACCATCAAAA
TCAATCATTTGAACTTAGGAAGTGGACAAAAAGTAGTTCTTACCTAT
GATGTACGTTTAAAAGATAACTATATAAGTAACAAATTTTACAATAC
AAATAATCGTACAACGCTAAGTCCGAAGAGTGAAAAAGAACCAAAT

FIG. 1 CONT'D

ACTATTCGTGATTTCCCAATTCCCAAAATTCGTGATGTTCGTGAGTTT
CCGGTACTAACCATCAGTAATCAGAAGAAAATGGGTGAGGTTGAATT
TATTAAAGTTAATAAAGACAAACATTCAGAATCGCTTTTGGGAGCTA
AGTTTCAACTTCAGATAGAAAAAGATTTTTCTGGGTATAAGCAATTT
GTTCCAGAGGGAAGTGATGTTACAACAAAGAATGATGGTAAAATTTA
TTTTAAAGCACTTCAAGATGGTAACTATAAATTATATGAAATTTCAA
GTCCAGATGGCTATATAGAGGTTAAAACGAAACCTGTTGTGACATTT
ACAATTCAAAATGGAGAAGTTACGAACCTGAAAGCAGATCCAAATG
CTAATAAAAATCAAATCGGGTATCTTGAAGGAAATGGTAAACATCTT
ATTACCAACACTCCCAAACGCCCACCAGGTGTTTTTCCTAAAACAGG
GGGAATTGGTACAATTGTCTATATATTAGTTGGTTCTACTTTTATGAT
ACTTACCATTTGTTCTTTCCGTCGTAAACAATTGTAA

MMIVNNGYLEGRKMKKRQKIWRGLSVTLLILSQIPFGILVQGETQDTNQ
ALGKVIVKKTGDNATPLGKATFVLKNDNDKSETSHETVEGSGEATFENI
KPGDYTLREETAPIGYKKTDKTWKVKVADNGATIIEGMDADKAEKRKE
VLNAQYPKSAIYEDTKENYPLVNVEGSKVGEQYKALNPINGKDGRREIA
EGWLSKKNPGVNDLDKNKYKIELTVEGKTTVETKELNQPLDVVVLLDN
SNSMNNERANNSQRALKAGEAVEKLIDKITSNKDNRVALVTYASTIFDG
TEATVSKGVADQNGKALNDSVSWDYHKTTFTATTHNYSYLNLTNDAN
EVNILKSRIPKEAEHINGDRTLYQFGATFTQKALMKANEILETQSSNARK
KLIFHVTDGVPTMSYAINFNPYISTSYQNQFNSFLNKIPDRSGILQEDFIIN
GDDYQIVKGDGESFKLFSDRKVPVTGGTTQAAYRVPQNQLSVMSNEGY
AINSGYIYLYWRDYNWVYPFDPKTKKVSATKQIKTHGEPTTLYFNGNIR
PKGYDIFTVGIGVNGDPGATPLEAEKFMQSISSKTENYTNVDDTNKIYDE
LNKYFKTIVEEKHSIVDGNVTDPMGEMIEFQLKNGQSFTHDDYVLVGND
GSQLKNGVALGGPNSDGGILKDVTVTYDKTSQTIKINHLNLGSGQKVVL
TYDVRLKDNYISNKFYNTNNRTTLSPKSEKEPNTIRDFPIPKIRDVREFPV
LTISNQKKMGEVEFIKVNKDKHSESLLGAKFQLQIEKDFSGYKQFVPEGS
DVTTKNDGKIYFKALQDGNYKLYEISSPDGYIEVKTKPVVTFTIQNGEVT
NLKADPNANKNQIGYLEGNGKHLITNTPKRPPGVFPKTGGIGTIVYILVG
STFMILTICSFRRKQL*

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Clone 141

ATGAATAGAAAAGTTGAGGAAAAAATGGCTGGGAATCGTAATAACG
ATATGAATGTCTATTGTTCATTTTGTGGCAAAAGCCAAGATGAAGTA
AAAAAAATTATTGCAGGTAATGGTGTTTTCATTTGTAATGAATGTGTG
GCCTTATCACAAGAAATTATTAAGGAAGAATTAGCTGAGGAAGTACT

FIG. 1CONT'D

GGCTCATTTAGCAGAAGTACCAAAACCTAAGGAACTATTAGAAATAT
TAAATCAATATGTTGTAGGGCAAGATCGTGCTAAACGTGCTTTAGCA
GTTGCTGTCTACAATCATTACAAGCGTGTTAGTTATACCGAGAGTAGT
GACGATGATGTAGATTTGCAAAAATCCAACATTTTGATGATTGGTCC
AACTGGCTCAGGAAAAACCTTCTTAGCACAAACACTGGCTAAAAGCC
TTAATGTACCGTTTGCTATTGCAGATGCGACTTCATTGACCGAAGCAG
GATACGTTGGAGAAGATGTTGAGAATATTCTTCTTAAATTGATTCAA
GCTGCTGATTATAATGTCGAACGTGCTGAGCGTGGTATTATCTACGTT
GATGAAATAGATAAAATTGCTAAGAAAGGCGAAAATGTTTCTATCAC
ACGTGATGTGTCTGGTGAAGGTGTACAGCAAGCCCTTCTTAAAATTA
TTGAGGGTACGGTAGCAAGTGTTCCCCCACAGGGTGGGCGTAAACAT
CCTAACCAAGAAATGATTCAAATTAATACCAAGAACATCCTTTTTATT
GTCGGTGGTGCTTTTGATGGTATTGAAGACCTTGTGAAGCAACGTTTA
GGCGAAAAAGTTATTGGTTTTGGACAGACAAGCCGTAAAATTGATGA
CAACGCTTCTTATATGCAAGAGATAATTTCTGAGGATATTCAAAAGT
TTGGACTGATTCCAGAGTTTATTGGCCGTTTACCAGTAGTTGCAGCGT
TAGAACTTCTTACTGCAGAAGATCTGGTTCGTATTCTGACAGAACCA
CGCAATGCTTTGGTTAAACAATACCAAACCTTATTATCTTATGATGGT
GTAGAATTGGAATTTGACCAGGATGCTCTATTGGCTATCGCTGATAA
GGCTATCGAGCGCAAGACTGGTGCACGTGGTTTACGTTCTATTATTG
AAGAAACGATGCTTGATATCATGTTTGAAATTCCAAGCCAAGAAGAT
GTAACAAAAGTTCGTATCACAAAGGCTGCTGTTGAGGGTACTGACAA
GCCTGTTTTAGAGACGGCTTAG

MNRKVEEKMAGNRNNDMNVYCSFCGKSQDEVKKIIAGNGVFICNECV
ALSQEIIKEELAEEVLAHLAEVPKPKELLEILNQYVVGQDRAKRALAVA
VYNHYKRVSYTESSDDDVDLQKSNILMIGPTGSGKTFLAQTLAKSLNVP
FAIADATSLTEAGYVGEDVENILLKLIQAADYNVERAERGIIYVDEIDKIA
KKGENVSITRDVSGEGVQQALLKIIEGTVASVPPQGGRKHPNQEMIQINT
KNILFIVGGAFDGIEDLVKQRLGEKVIGFGQTSRKIDDNASYMQEIISEDI
QKFGLIPEFIGRLPVVAALELLTAEDLVRILTEPRNALVKQYQTLLSYDG
VELEFDQDALLAIADKAIERKTGARGLRSIIEETMLDIMFEIPSQEDVTKV
RITKAAVEGTDKPVLETA*

ID-71

Clone 3-20

ATGAAAAGATTACATAAACTGTTTATAACCGTAATTGCTACATTAGG
TATGTTGGGGGTAATGACCTTTGGTCTTCCAACGCAGCCGCAAAACG
TAACGCCGATAGTACATGCTGATGTCAATTCATCTGTTGATACGAGC
CAGGAATTTCAAAATAATTTAAAAAATGCTATTGGTAACCTACCATT
TCAATATGTTAATGGTATTTATGAATTAAATAATAATCAGACAAATTT
AAATGCTGATGTCAATGTTAAAGCGTATGTTCAAAATACAATTGACA

FIG. 1CONT'D

ATCAACAAAGACTATCAACTGCTAATGCAATGCTTGATAGAACCATT
CGTCAATATCAAAATCGCAGAGATACCACTCTTCCCGATGCAAATTG
GAAACCATTAGGTTGGCATCAAGTAGCTACTAATGACCATTATGGGC
ATGCAGTCGACAAGGGGCATTTAATTGCCTATGCTTTAGCTGGAAAT
TTCAAAGGTTGGGATGCTTCCGTGTCAAATCCTCAAAATGTTGTCACA
CAAACAGCTCATTCCAACCAATCAAATCAAAAAATCAATCGTGGACA
AAATTATTATGAAAGCTTAGTTCGTAAGGCGGTTGACCAAAACAAAC
GTGTTCGTTACCGTGTAACTCCATTGTACCGTAATGATACTGATTTAG
TTCCATTTGCAATGCACCTAGAAGCTAAATCACAAGATGGCACATTA
GAATTTAATGTTGCTATTCCAAACACACAAGCATCATACACTATGGA
TTATGCAACAGGAGAAATAACACTAAATTAA

MKRLHKLFITVIATLGMLGVMTFGLPTQPQNVTPIVHADVNSSVDTSQE
FQNNLKNAIGNLPFQYVNGIYELNNNQTNLNADVNVKAYVQNTIDNQQ
RLSTANAMLDRTIRQYQNRRDTTLPDANWKPLGWHQVATNDHYGHAV
DKGHLIAYALAGNFKGWDASVSNPQNVVTQTAHSNQSNQKINRGQNY
YESLVRKAVDQNKRVRYRVTPLYRNDTDLVPFAMHLEAKSQDGTLEFN
VAIPNTQASYTMDYATGEITLN*

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Clone 13

ATGAAAAACTATCGAAAACTTATTGTACTACTACTTCTAATCTTTTTT
GCCATTTTTATGGGAGCATATGCTTACACGCATATTGTTGAAAAAAG
ATCCCTAACTAGCAATACTATTGAAAAAACTCTACCTGTGGTAAATC
AGATTAAGCCTCAAACCATTAAAGAATACCAAAATTACTTAACTAAG
GTAGCTAAACGTAATGTTCTTCCTGTAGACATTCCTCAGGCATTAAAT
AATGAAAAGGTAGAAATTACTGCTACTGATGGCATGCAAACATTCAC
TTGGAATGATAAAAATAATCCTAAGCAAAAGGTTATCTTCTATGTTC
ATGGAGGATCATATATCCATCAAGCTTCCGAATTACAATATATTTTTG
TCAATAAACTAGCTAAAAAATTAGATGCAAAAGTTGTCTTTCCTATTT
ACCCTAAAGCTCCTACATATAATTATAGTGATGCTATCCCCAAAATTA
AAAAATTATACCAAAATACATTAGCTAGCGTCACATCTCACAAACAG
ATTATCCTAGTAGGTGAAAGTGCAGGCGGAGGCCTTGCTTTAGGTAT
TGCTGATAACCTTGCACGGAGCATATCAAACAACCAAAAGAAATTAT
TTTAA

MKNYRKLIVLLLLIFFAIFMGAYAYTHIVEKRSLTSNTIEKTLPVVNQIKP
QTIKEYQNYLTKVAKRNVLPVDIPQALNNEKVEITATDGMQTFTWNDK
NNPKQKVIFYVHGGSYIHQASELQYIFVNKLAKKLDAKVVFPIYPKAPT
YNYSDAIPKIKKLYQNTLASVTSHKQIILVGESAGGGLALGIADNLARSIS
NNQKKLF*

FIG. 1CONT'D

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Clone 2-19

TTGATTCTAATAACTTCCTATGGGATAATATCTTTATCACAAAAATTG
AGGGAATTTATTATGAAGTTAAAACATATTGTCTTAGGATTAGCCTTA
ACAACACTTTTAGGAGTCACATTTAGTAATCAAGAAGTTTCAGCAAG
CTCAACTTCAAGTAAAGTTGTTAAAGTTGGTGTTATGACCTTTTCTGA
CACTGAAAAAGCACGTTGGGATAAAATTGAAAAGCTAGTAGGTGAT
AAAGCTAAAATCAAATTTACAGAATTTACAGATTATACACAACCAAA
TCAAGCGACAGCCAATAAGGATGTGGATATTAATGCCTTTCAACATT
ACAATTTCTTAGAAAACTGGAATAAGGAAAATAAGAAAAACTTAATT
CCACTTGAAAAGACTTACTTAGCTCCAATTCGTATCTATTCTGAGAAG
GTAAAATCTCTTAAAAAATTGAAAAAAGGAGCCACTATTGCAATTCC
AAATGATGCAACAAATGGTAGCCGTGCATTGTATGTCCTTCAGTCAG
CAGGTTTAATCAAATTGAATGTTTCTGGTAAGAAGGTTGCAACAGTT
GCTAATATCACATCTAATAAAAAGGATATTAATATTCAGGAGTTAGA
TGCGAGTCAAACACCACGTGCACTCAAAGATGTAGATGCAGCTATTA
TTAATAATACATACATTGAGCAAGCTAATTTAAAACCTTCAGATGCT
ATCTTTGTTGAGAAATCAGATAAAAATTCAAAACAATGGATTAATAT
CATTGCGGGACGTAAAAATTGGAAAAAGCAAAAGAACGCTAAAGCT
ATCCAAGCTATCTTGGATGCTTATCACACAGATGAAGTGAAAAAAGT
TATCAAAGATACTTCAGCTGATATTCCACAATGGTAA

MILITSYGIISLSQKLREFIMKLKHIVLGLALTTLLGVTFSNQEVSASSTSS
KVVKVGVMTFSDTEKARWDKIEKLVGDKAKIKFTEFTDYTQPNQATAN
KDVDINAFQHYNFLENWNKENKKNLIPLEKTYLAPIRIYSEKVKSLKKL
KKGATIAIPNDATNGSRALYVLQSAGLIKLNVSGKKVATVANITSNKKDI
NIQELDASQTPRALKDVDAAIINNTYIEQANLKPSDAIFVEKSDKNSKQW
INIIAGRKNWKKQKNAKAIQAILDAYHTDEVKKVIKDTSADIPQW*

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Clone 3-6

ATGTCAAATCAATATGATTATATCGTTATTGGTGGAGGTAGTGCAGG
CAGTGGTACCGCTAATAGGGCAGCCATGTATGGAGCAAAAGTCCTGT
TAATTGAAGGTGGACAAGTAGGTGGAACTTGTGTTAACTTAGGTTGT
GTACCTAAGAAAATCATGTGGTATGGTGCACAAGTTTCTGAGACACT
CCATAAGTATAGTTCAGGTTATGGTTTTGAAGCCAATAATCTTAGTTT
TGATTTTACTACTCTAAAAGCTAATCGCGATGCTTACGTGCAGCGGTC
TAGACAGTCGTATGCCGCTAATTTTGAGCGTAATGGGGTCGAAAAGA

FIG. 1 CONT'D

TTGATGGATTTGCTCGTTTTATTGATAACCATACTATTGAAGTGAATG
GTCAGCAATATAAAGCTCCTCACATTACTATTGCAACAGGTGGACAC
CCTCTTTACCCTGATATTATTGGAAGTGAACTTGGTGAGACTTCTGAT
GATTTTTTTGGATGGGAGACCTTACCAAATTCTATATTGATTGTTGGG
GCGGGCTATATCGCGGCAGAACTTGCTGGAGTGGTTAATGAATTAGG
CGTTGAAACCCATCTTGCATTTAGAAAAGACCATATTCTACGCGGAT
TTGATGACATGGTAACAAGTGAGGTTATGGCTGAAATGGAGAAATCA
GGTATCTCTTTACATGCTAACCATGTACCTAAATCTCTTAAACGCGAT
GAAGGTGGCAAGTTGATTTTTGAAGCTGAAAATGGAAAAACGCTTGT
CGTTGATCGTGTAATATGGGCTATCGGCCGTGGACCAAATGTAGACA
TGGGACTTGAAAATACCGATATTGTTTTAAATGATAAAGATTATATC
AAAACAGATGAATTTGAGAATACTTCTGTAGATGGCGTGTATGCTAT
TGGAGATGTTAATGGGAAAATTGCCTTGACACCGGTAGCAATTGCAG
CAGGTCGTCGCTTATCAGAAAGACTTTTTAATCATAAAGATAACGAA
AAATTAGATTACCATAATGTACCTTCAGTTATTTTTACTCACCCTGTA
ATTGGGACGGTAGGACTTTCAGAAGCAGCAGCTATCGAGCAATTTGG
AAAAGATAATATCAAAGTCTATACATCAACTTTTACCTCTATGTATAC
GGCTGTTACCAGTAATCGCCAAGCAGTTAAGATGAAGCTCATAACCC
TAGGAAAAGAGGAAAAAGTTATTGGGCTTCATGGTGTTGGTTATGGT
ATTGATGAAATGATTCAAGGTTTTTCAGTTGCTATCAAAATGGGGGC
TACTAAAGCAGACTTTGATGATACTGTTGCTATTCACCCAACTGGATC
TGAGGAATTTGTTACAATGCGCTAA

MSNQYDYIVIGGGSAGSGTANRAAMYGAKVLLIEGGQVGGTCVNLGC
VPKKIMWYGAQVSETLHKYSSGYGFEANNLSFDFTTLKANRDAYVQRS
RQSYAANFERNGVEKIDGFARFIDNHTIEVNGQQYKAPHITIATGGHPLY
PDIIGSELGETSDDFFGWETLPNSILIVGAGYIAAELAGVVNELGVETHLA
FRKDHILRGFDDMVTSEVMAEMEKSGISLHANHVPKSLKRDEGGKLIFE
AENGKTLVVDRVIWAIGRGPNVDMGLENTDIVLNDKDYIKTDEFENTSV
DGVYAIGDVNGKIALTPVAIAAGRRLSERLFNHKDNEKLDYHNVPSVIF
THPVIGTVGLSEAAAIEQFGKDNIKVYTSTFTSMYTAVTSNRQAVKMKLI
TLGKEEKVIGLHGVGYGIDEMIQGFSVAIKMGATKADFDDTVAIHPTGS
EEFVTMR*

ID-75

Clone 3-51

ATGAGTATCAAAAAAAGTGTGATTGGTTTTTGCCTCGAAGCTGCAGC
ATTATCAATGTTTGCTTGTGTAGACAGTAGTCAATCTGTTATGGCTGC

FIG. 1CONT'D

CGAGAAGGATAAAGTCGAAATTACGTGGTGGGCTTTTCCAACCTTTA
CTCAAGAAAAGGCTAAGGATGGAGTAGGTACTTATGAGAAAAAAGT
CATCAAGGCTTTTGAAAAGAAAAATCCTAATATAAAAGTAAAACTAG
AGACAATTGATTTCACATCTGGACCTGAAAAAATCACTACAGCAATT
GAAGCAGGGACAGCACCTGATGTGCTTTTTGATGCACCAGGGCGAAT
TATTCAATATGGTAAAAATGGTAAATTAGCAGATTTGAATGATTTATT
TACAGACCAATTTATTAAGGATGTCAATAATAAGAACATCATTCAAG
CTTCTAAGTCTGGCGATAAAGCCTACATGTATCCAATAAGTTCTGCCC
CATTTTATATGGCGTTCAATAAAAAAATGCTTAAAGATGCAGGAGTT
TTGAAACTTGTAAAAGAAGGTTGGACTACTAGTGATTTTGAAAAAGT
ACTAAAAGCACTAAAAAATAAAGGCTATACACCAGGTTCATTCTTTG
CAAACGGGCAAGGAGGAGATCAAGGACCACGTGCATTTTTTGCTAAT
CTTTATAGTGCTCCAATAACAGATAAAGAAGTAACAAAATATACCAC
TGACACTAAAAATTCTGTAAAATCAATGAAAAAAATAGTTGAATGGA
TTAAGAAAGGCTACTTGATGAATGGGTCTCAGTATGATGGCTCAGCT
GACATTCAAAACTTCGCCAATGGACAAACTGCTTTCACTATCCTATG
GGCTCCAGCTCAACCAAAAACTCAAGCAAAATTATTAGAGTCAAGTA
AAGTGGATTACCTTGAAGTGCCATTCCCATCAGAAGATGGAAAACCA
GATTTAGAATACCTTGTTAATGGTTTTGCGGTCTTTAATAATAAAGAT
GAAAACAAAGTAAAAGCCTCTAAGAAATTTATCACTTTTATTGCTGA
TGATAAAAAATGGGGACCAAAAGATGTTATACGTACAGGTGCTTTCC
CAGTTAGAACATCATTTGGGGATCTTTATAAAGGTGATAAACGTATG
ATGAAGATTTCAAAATGGACTCAATATTATTCACCATATTACAACAC
TATCGATGGATTTTCTGAAATGAGAACCTTATGGTTCCCAATGGTTCA
ATCTGTATCCAATGGTGATGAAAAACCAGCAGATGCTTTGAAAGACT
TTACTCAAAAAGCAAATGATACCATTAAAAAAGCAGCTAAATAA

MSIKKSVIGFCLEAAALSMFACVDSSQSVMAAEKDKVEITWWAFPTFTQ
EKAKDGVGTYEKKVIKAFEKKNPNIKVKLETIDFTSGPEKITTAIEAGTAP
DVLFDAPGRIIQYGKNGKLADLNDLFTDQFIKDVNNKNIIQASKSGDKA
YMYPISSAPFYMAFNKKMLKDAGVLKLVKEGWTTSDFEKVLKALKNK
GYTPGSFFANGQGGDQGPRAFFANLYSAPITDKEVTKYTTDTKNSVKSM
KKIVEWIKKGYLMNGSQYDGSADIQNFANGQTAFTILWAPAQPKTQAK
LLESSKVDYLEVPFPSEDGKPDLEYLVNGFAVFNNKDENKVKASKKFIT
FIADDKKWGPKDVIRTGAFPVRTSFGDLYKGDKRMMKISKWTQYYSPY
YNTIDGFSEMRTLWFPMVQSVSNGDEKPADALKDFTQKANDTIKKAAK
*

ID-76 (Same as ID-39)

Clone 3-56
ATGAGGAAACGTTTTTCCTTGCTAAATTTTATTGTTGTTACTTTTATTT
TCTTTTTCTTTATTCTTTTTCCGCTTTTTAAGGCCAAAGATTGTCAGGT

FIG. 1 CONT'D

TGTTTATGCAAGTTTTCAAGGAGATCATTGGGACATTTGTAACGCATT
TGATTTTCCGTATTTACATCGCTTTGATCTCATTAAAGGTAAAGAAAA
TCAACTTTACTTTATAGGTTGTACAATTGCTAACAGTAAAGCCTACAC
TGAGGATTGGAGTGATAAAGGCCGAATTTTTGTTGCTCGTTTTAATAC
TCAAAACCATACATTGGAAGGATTGCAACAATTGCCTCAAACTTTAT
TAAAAAATCATGGATACTATGCCATTCAGGATGAAGGATATTCATTG
ATTACTTCAGTAGAAGGGGTACTCAAACTCACTTATCCAGAATTTTCT
ACTACAGGCGACTGGCAATTAGAACGGCTTTTCGATGAGGAGACAAG
CGATGTGGTGAAAGTGGATATTAATCAGGATGGTAAGGATGAGTATG
TGATCATCCAAGGTTTTCATGGAGATCGTTTACGTATCTTCACTGAAG
ATTTCGGTCGAGAATTATTCCATTATCCTGAAAAAACCCCATTTGGTC
ACGCTATTTGGAGTGGTCGTTTACTTAATCAGACTTGTTTCGTATTCG
GGTGGCGATCAGAAAAAGCAGAATTAAGGCTTTTTCACTTTGTAGAT
GGGCACTTGGTTTCAGAATTAGTAGATGCAAAAGCAGCTTCTAGTAA
TGTCTTAGCTTTTGAAAAAGATGGAAAAGCTTATCTTTTCTCAGCCAA
TAACGGACGTGGCGAAGTTGCTCTTTATCAATTAGTAAAATAA

MRKRFSLLNFIVVTFIFFFFILFPLFKAKDCQVVYASFQGDHWDICNAFDF
PYLHRFDLIKGKENQLYFIGCTIANSKAYTEDWSDKGRIFVARFNTQNHT
LEGLQQLPQTLLKNHGYYAIQDEGYSLITSVEGVLKLTYPEFSTTGDWQ
LERLFDEETSDVVKVDINQDGKDEYVIIQGFHGDRLRIFTEDFGRELFHY
PEKTPFGHAIWSGRLLNQTCFVFGWRSEKAELRLFHFVDGHLVSELVDA
KAASSNVLAFEKDGKAYLFSANNGRGEVALYQLVK*

FIG. 1CONT'D

nucS1

Bgl II Eco RV

5'-cgagatctgatatctcacaaacagataacggcgtaaatag -3'

nucS2

Bgl II Sma I

5'-gaagatcttccccgggatcacaaacagataacggcgtaaatag -3'

nucS3

Bgl II Eco RV

5'-cgagatctgatatccatcacaaacagataacggcgtaaatag -3'

nucR

Bam HI

5'-cgggatccttatggacctgaatcagcgttgtc -3'

NucSeq

5'-ggatgctttgtttcaggtgtatc -3'

$pTREP_F$

5'-catgatatcggtacctcaagctcatatcattgtccggcaatggtgtgggcttttttgttttagcggataa caatttcacac -3'

$pTREP_R$

5'-gcggatcccccgggcttaattaatgtttaaacactagtcgaagatctcgcgaattctcctgtgtgaaatt gttatccgcta -3'

pUC_F

5'-cgccagggttttcccagtcacgac -3'

FIG. 2

V_R

5'-tcagggggcggagcctatg -3'

V_1

5'-tcgtatgttgtgtggaattgtg -3'

V_2

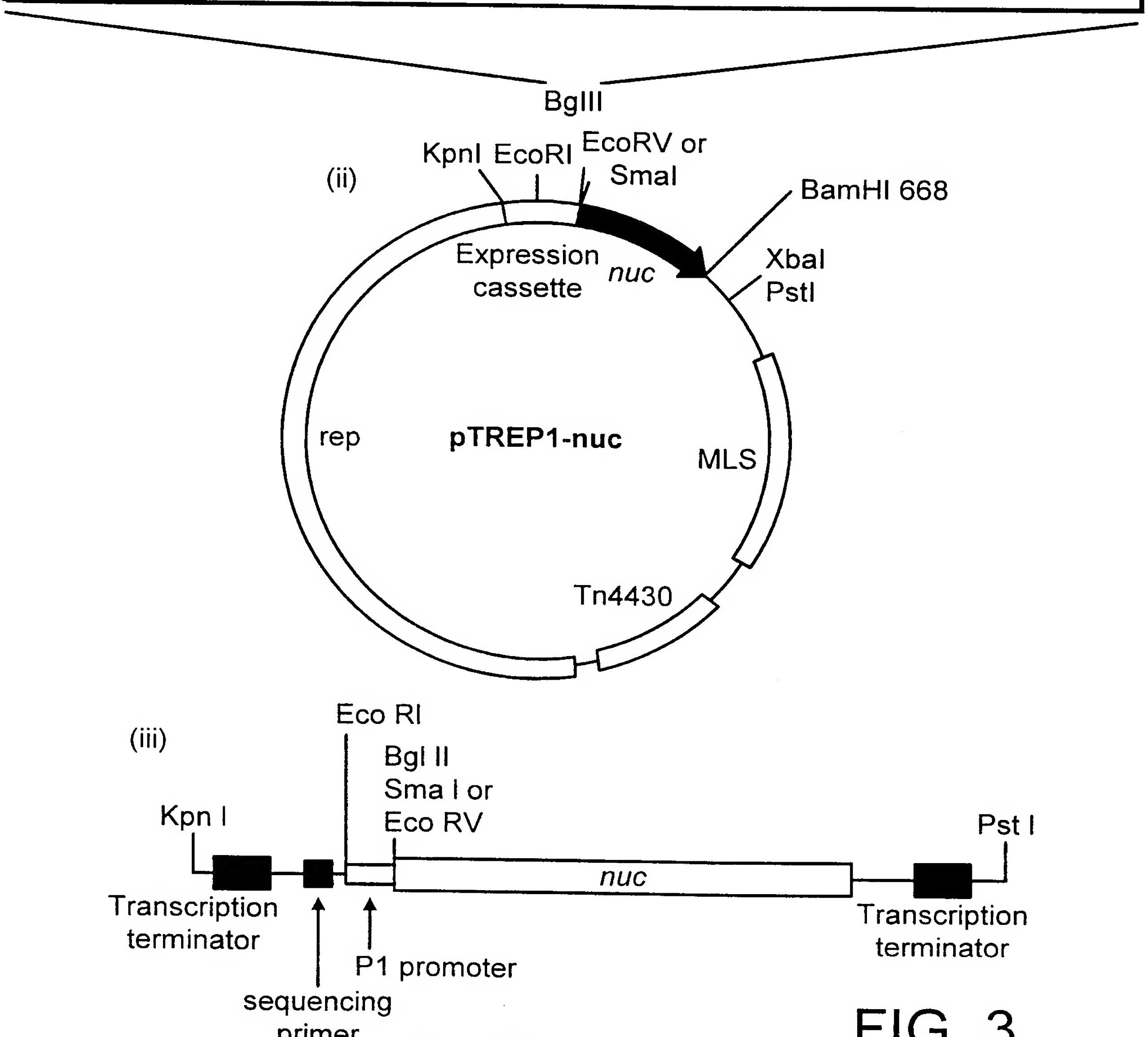
5'-tccggctcgtatgttgtgtggaattg -3'

pTREP-Nuc vectors allow cloning of genomic DNA into each frame with respect to the nuclease gene

(i)

```
pTREP1-nuc1  (EcoRV)  AAGTATCAGATCT--GATATC--TCACAAACAGATAACGGCGTAAAT  Frame=+1
                      :::::::::::::     ▲     ::::::::::::::::::::::::
                      :::::::::::::           ::::::::::::::::::::::::
pTREP1-nuc2  (Sma 1)  AAGTATCAGATCTTCCCCGGGA-TCACAAACAGATAACGGCGTAAAT  Frame=+2
                      :::::::::::::     ▲     ::::::::::::::::::::::::
                      :::::::::::::           ::::::::::::::::::::::::
pTREP1-nuc3  (EcoRV)  AAGTATCAGATCT--GATATCCATCACAAACAGATAACGGCGTAAAT  Frame=+3
                                        ▲     ::::::::::::::::::::::::
                                              ::::::::::::::::::::::::
Nuclease Gene                                 TCACAAACAGATAACGGCGTAAAT

Cloning site is indicated bt an arrow
```



The pTREP-nuc Cassette

FIG. 3

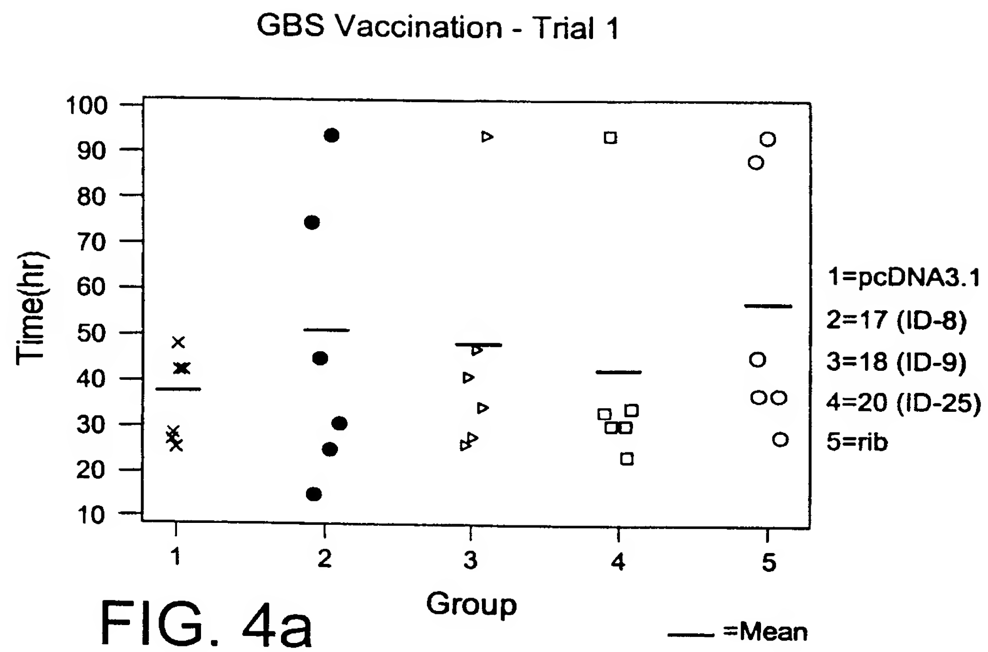

GBS Vaccination - Trial 1
Time(hr)
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
Group
1=pcDNA3.1
2=17 (ID-8)
3=18 (ID-9)
4=20 (ID-25)
5=rib
=Mean

FIG. 4a

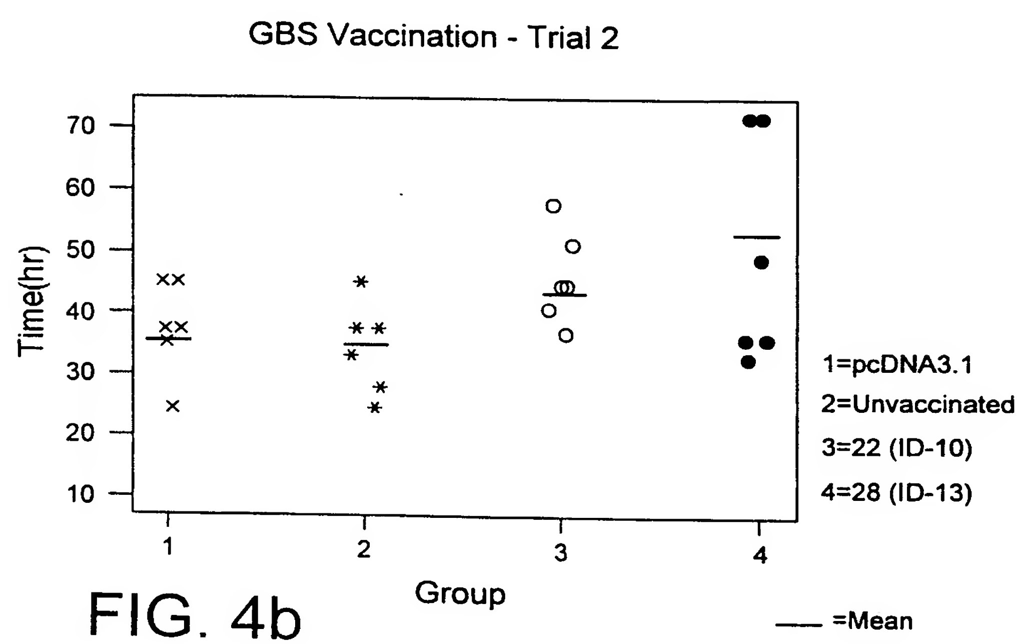

GBS Vaccination - Trial 2
Time(hr)
70
60
50
40
30
20
10
1
2
3
4
Group
1=pcDNA3.1
2=Unvaccinated
3=22 (ID-10)
4=28 (ID-13)
=Mean

FIG. 4b

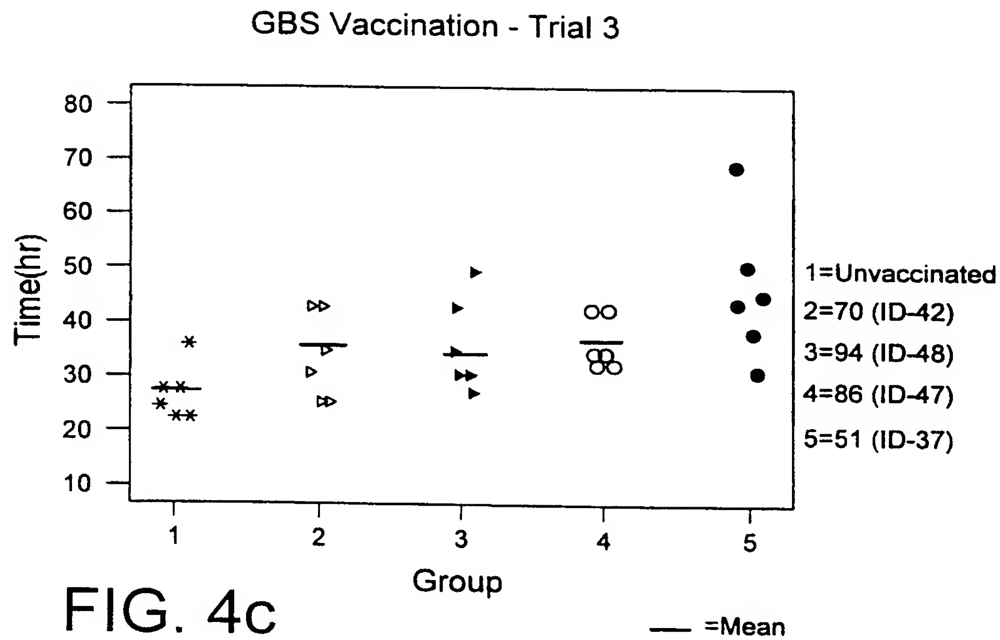

GBS Vaccination - Trial 3
Time(hr)
80
70
60
50
40
30
20
10
1
2
3
4
5
Group
1=Unvaccinated
2=70 (ID-42)
3=94 (ID-48)
4=86 (ID-47)
5=51 (ID-37)
=Mean

FIG. 4c

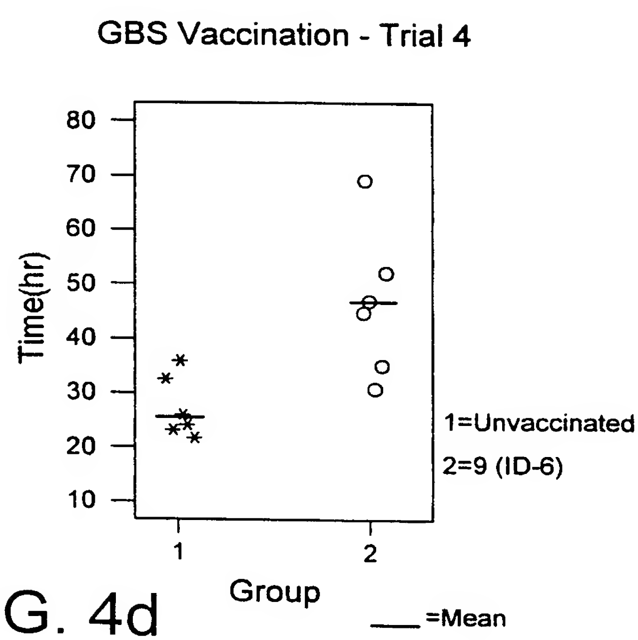

GBS Vaccination - Trial 4
Time(hr)
80
70
60
50
40
30
20
10
1
2
Group
1=Unvaccinated
2=9 (ID-6)
=Mean

FIG. 4d

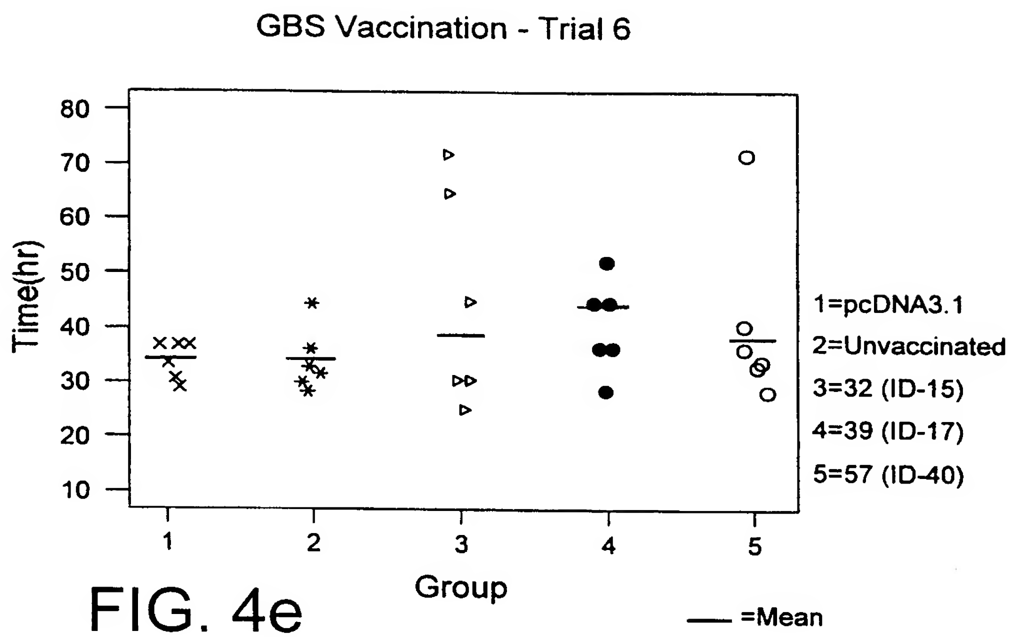

GBS Vaccination - Trial 6
Time(hr)
80
70
60
50
40
30
20
10
1
2
3
4
5
Group
1=pcDNA3.1
2=Unvaccinated
3=32 (ID-15)
4=39 (ID-17)
5=57 (ID-40)
=Mean

FIG. 4e

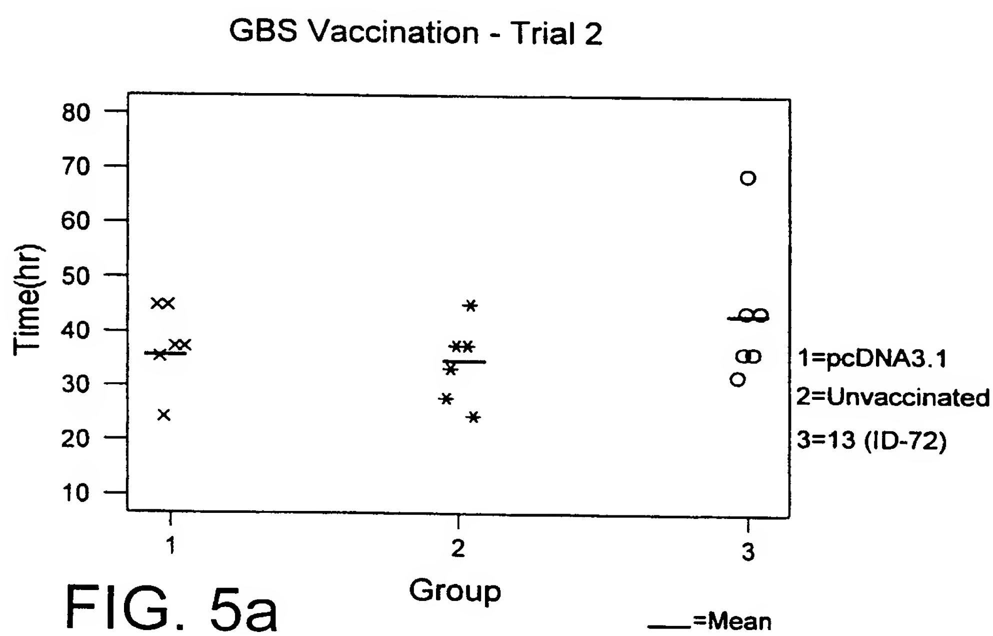

GBS Vaccination - Trial 2
Time(hr)
80
70
60
50
40
30
20
10
1
2
3
Group
1=pcDNA3.1
2=Unvaccinated
3=13 (ID-72)
=Mean

FIG. 5a

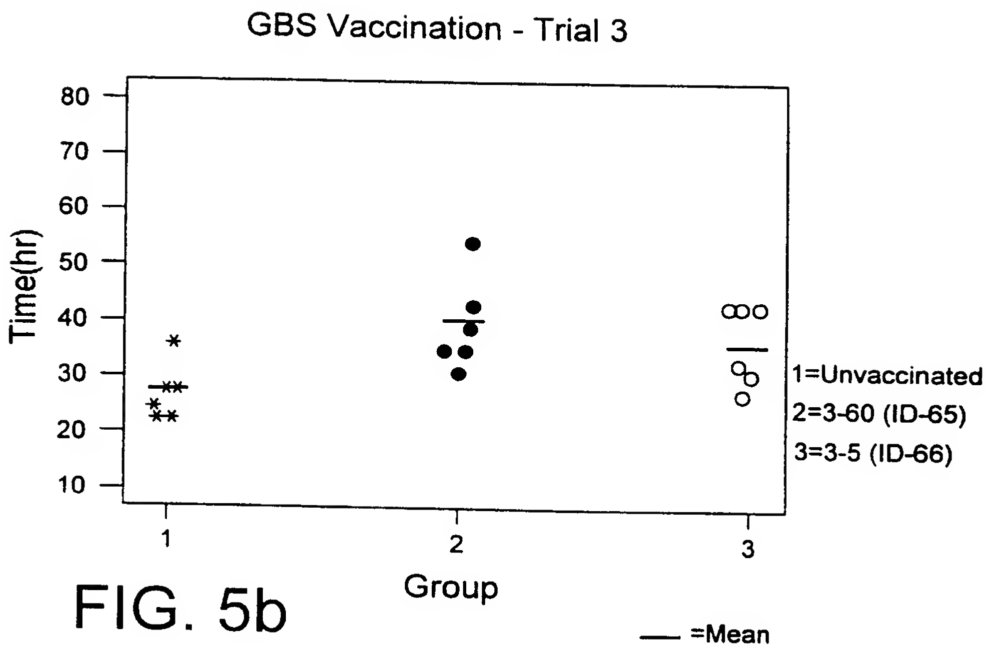

GBS Vaccination - Trial 3
Time(hr)
80
70
60
50
40
30
20
10
1
2
3
Group
1=Unvaccinated
2=3-60 (ID-65)
3=3-5 (ID-66)
=Mean

FIG. 5b

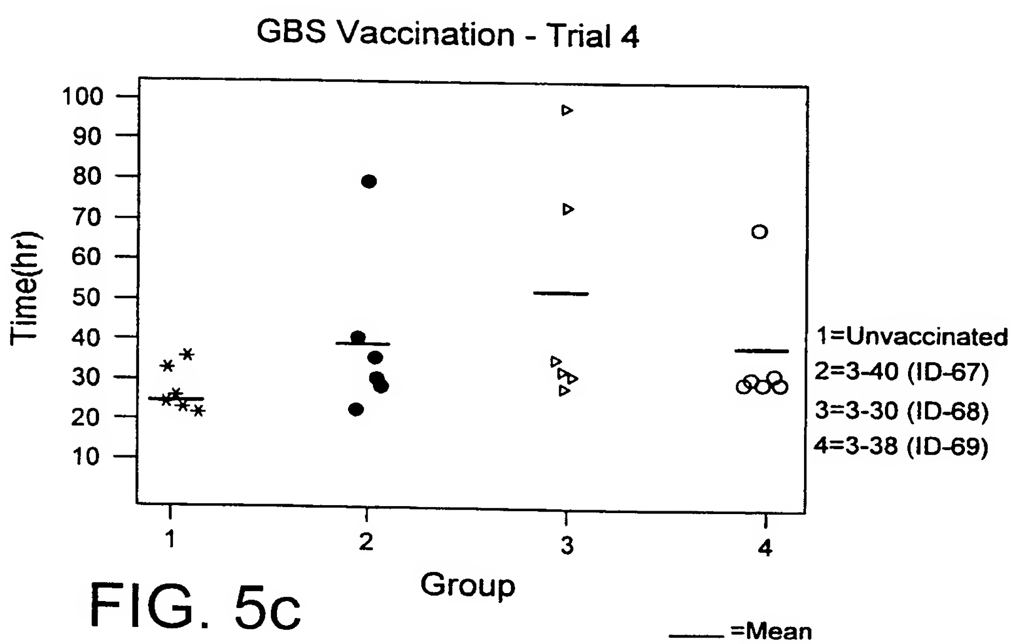

GBS Vaccination - Trial 4
Time(hr)
100
90
80
70
60
50
40
30
20
10
1
2
3
4
Group
1=Unvaccinated
2=3-40 (ID-67)
3=3-30 (ID-68)
4=3-38 (ID-69)
=Mean

FIG. 5c

GBS Vaccination - Trial 5

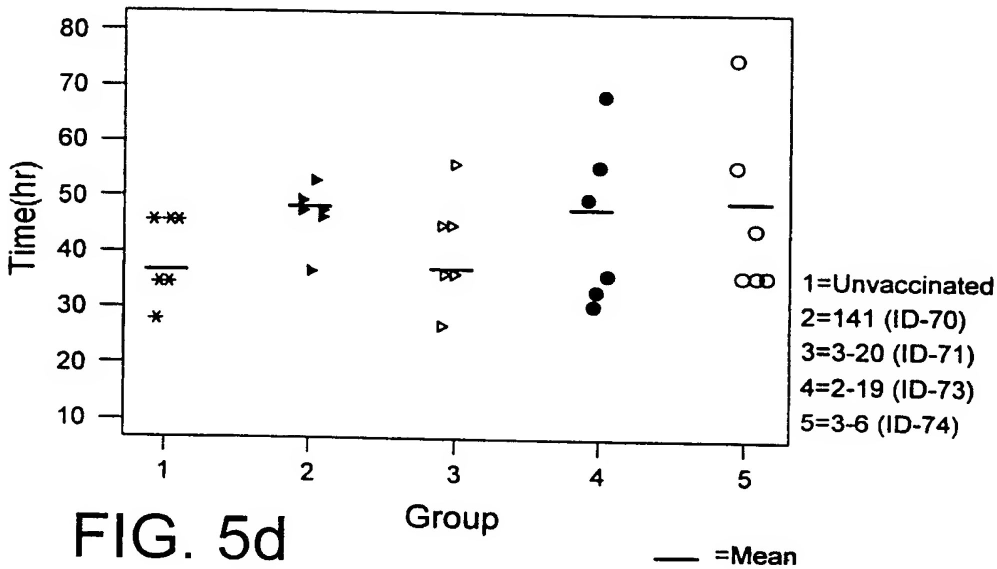


FIG. 5d

GBS Vaccination - Trial 6

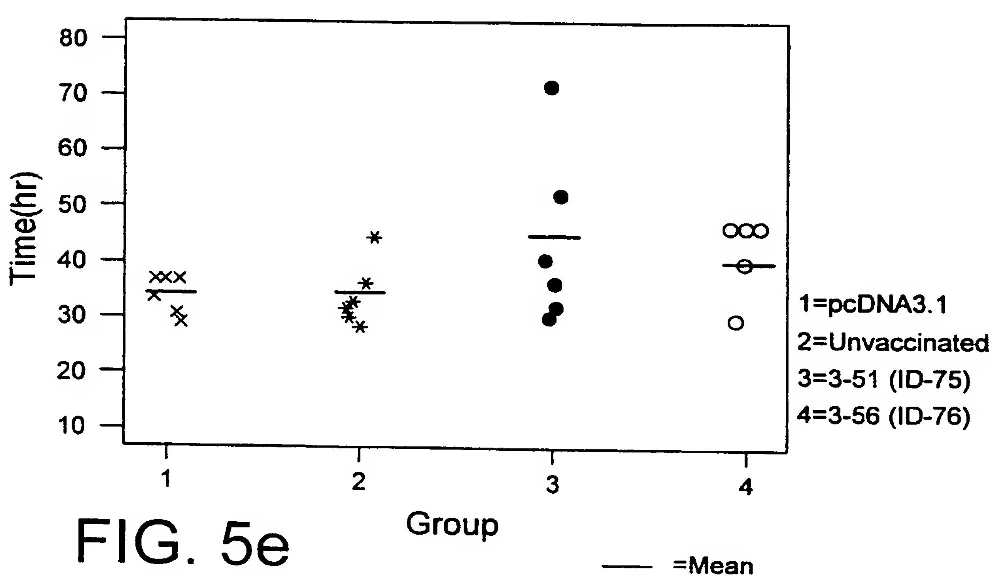


FIG. 5e

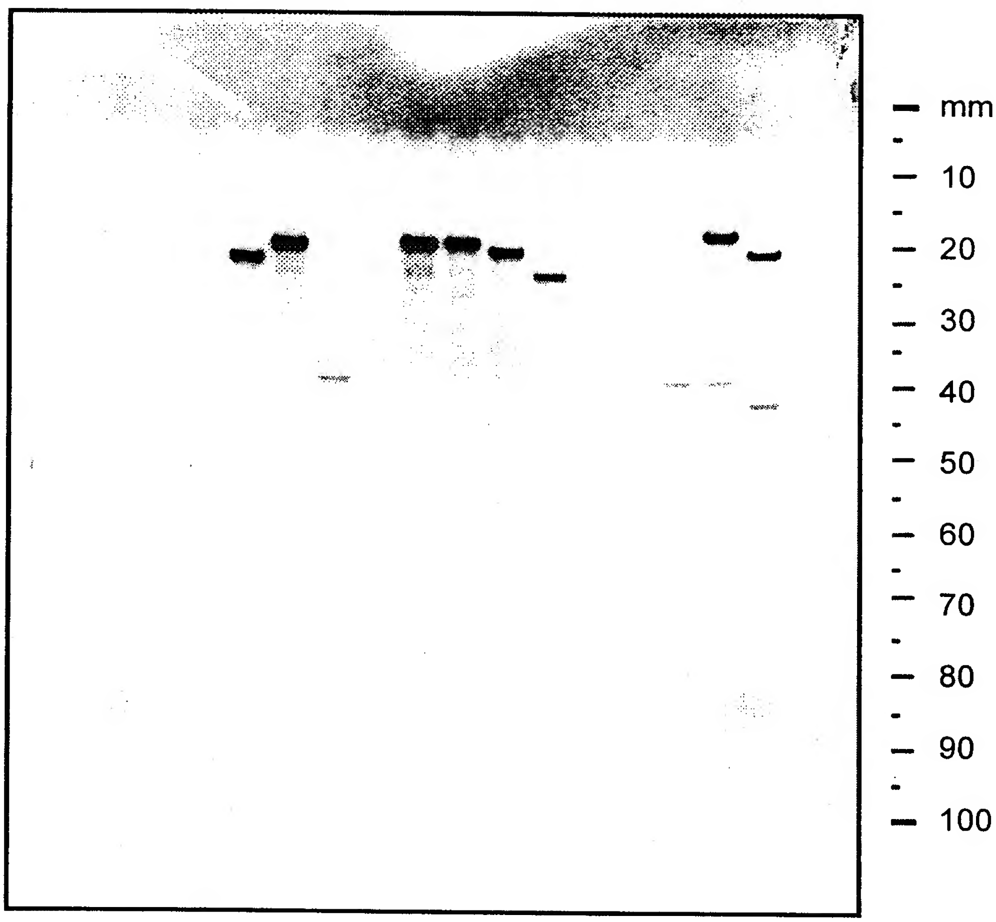


FIG. 6

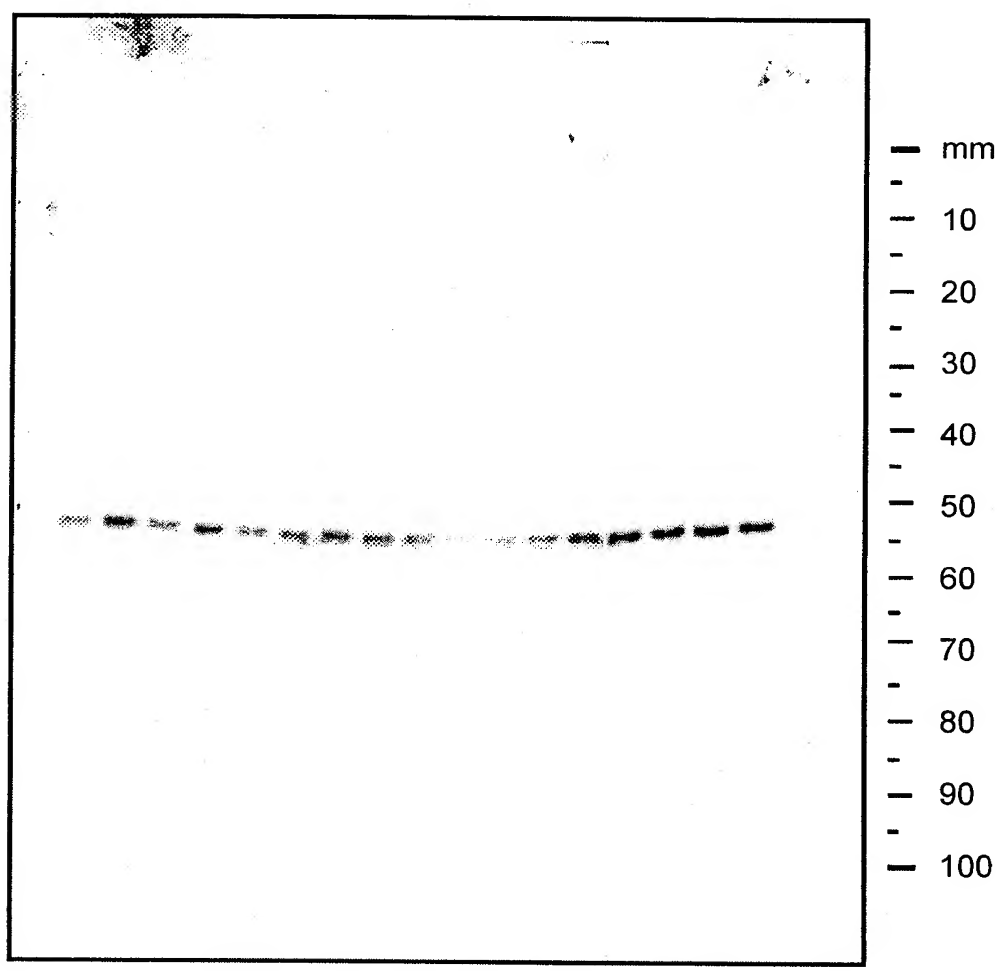


FIG. 7

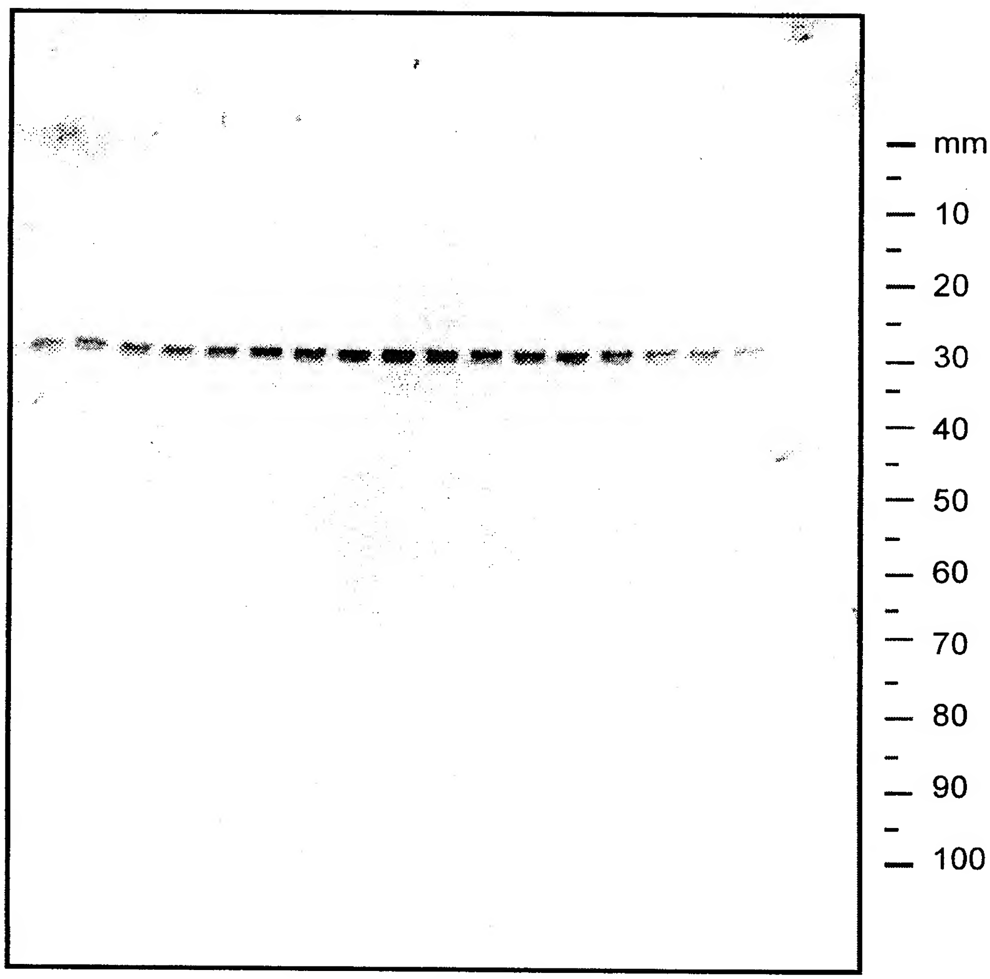


FIG. 8

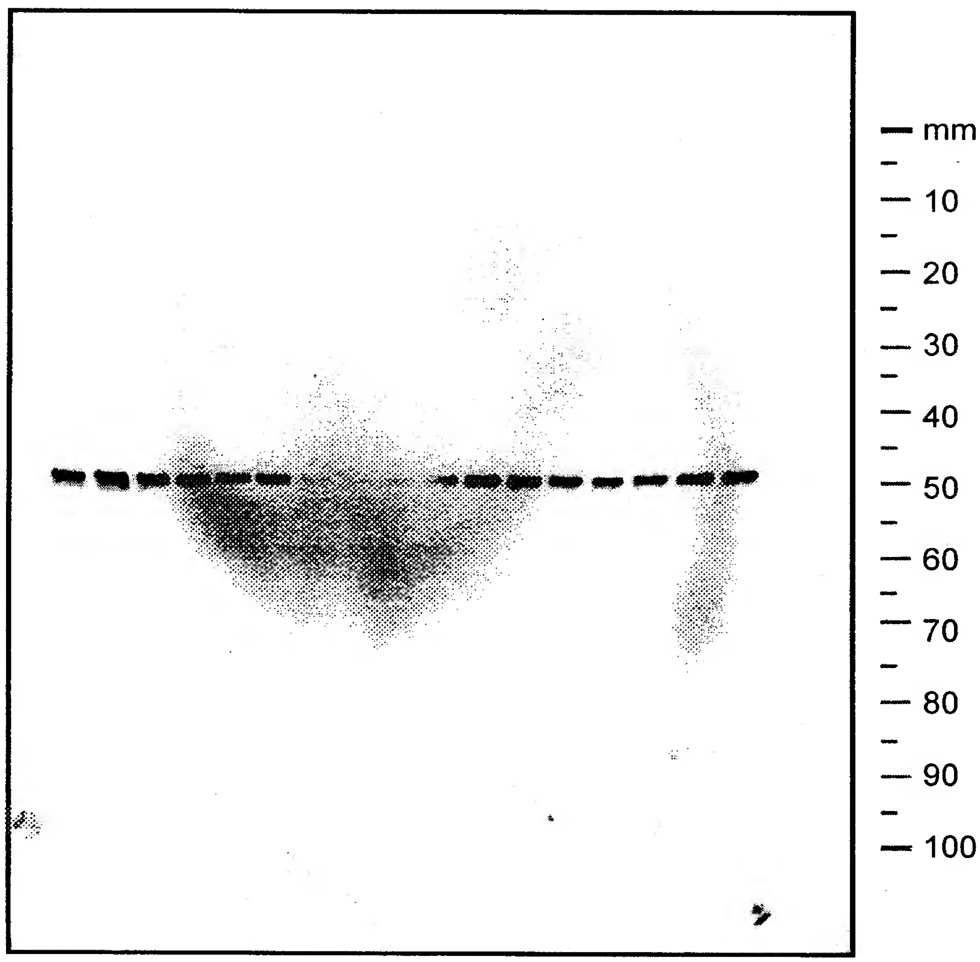


FIG. 9

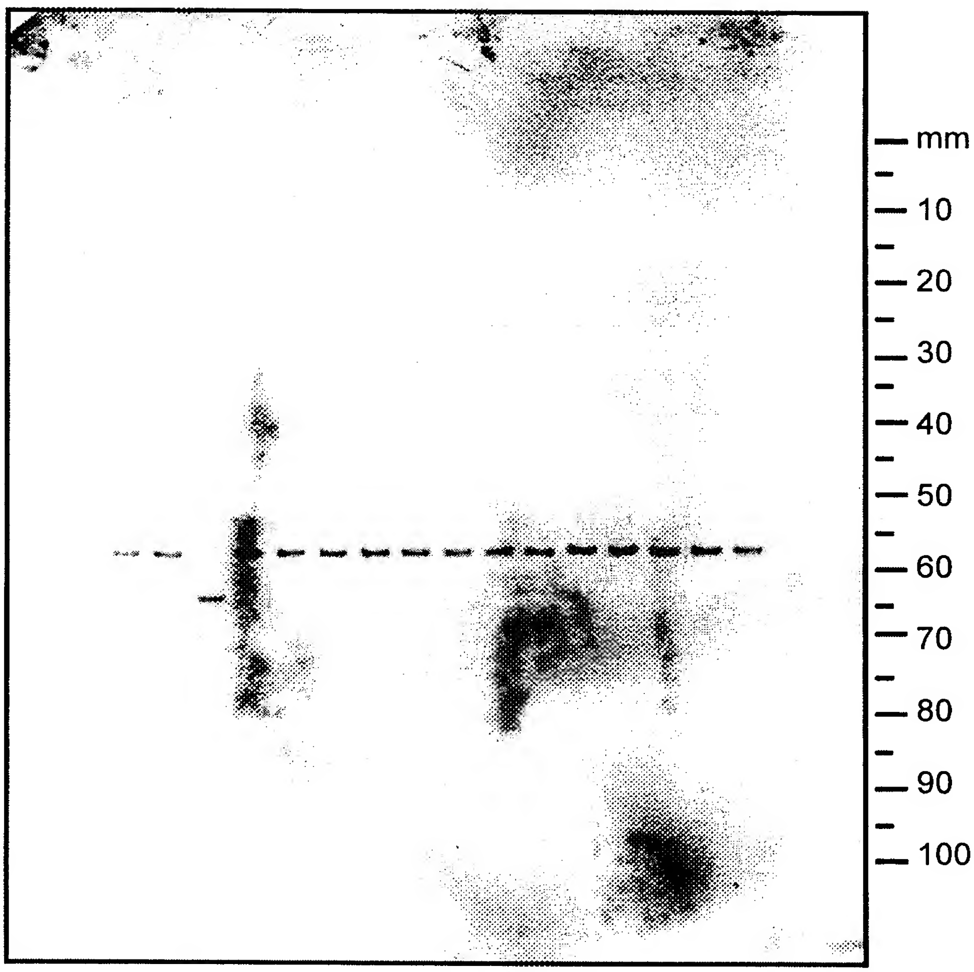


FIG. 10

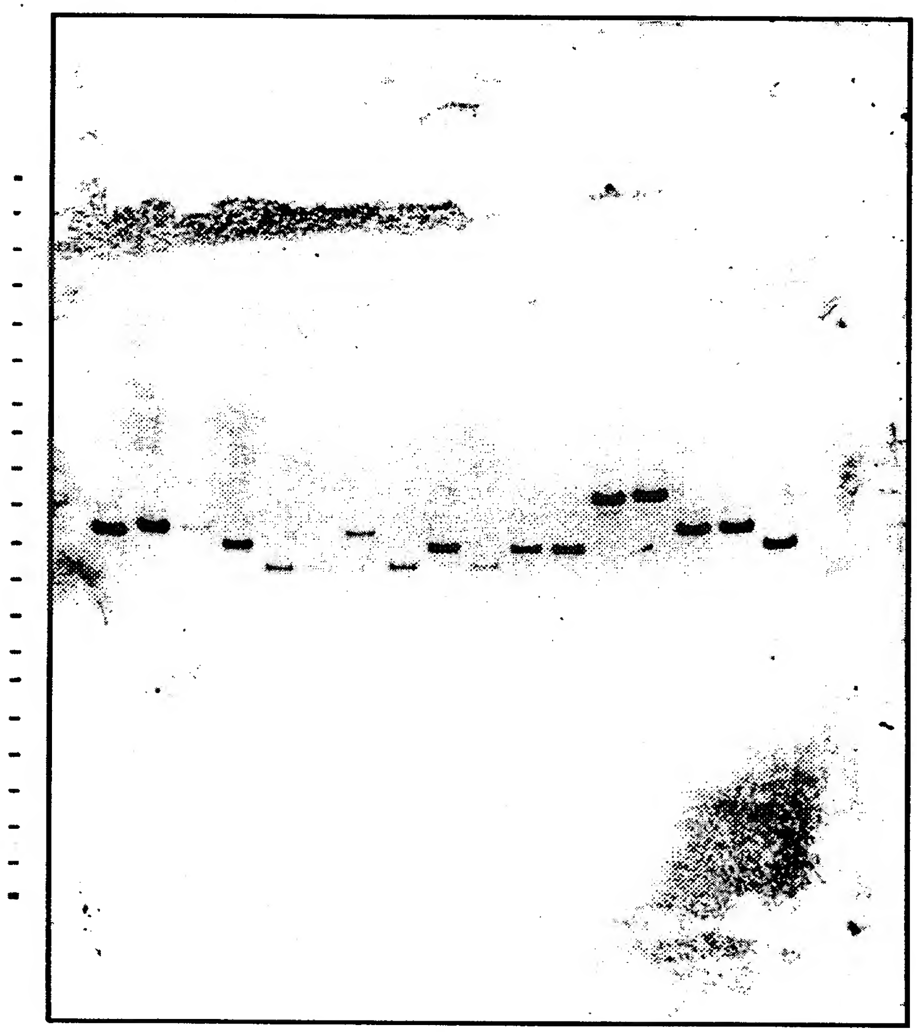


FIG. 11

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C12N 15/31, 15/74, 15/62, 15/10, 9/16, 1/19, 1/21, C07K 14/315, 16/12, A61K 31/70, 39/09, G01N 33/53, 33/68, C12Q 1/68

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(74) Agents: CHAPMAN, Paul, William et al.; Kilburn & Strode, 20 Red Lion Street, London WC1R 4PJ (GB).

(81) Designated States: CA, CN, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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(57) Abstract

Novel protein antigens from Group B *Streptococcus* are described, together with nucleic acid sequences encoding them. Their use in vaccines and screening methods is also described.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/02444

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C12N15/74 C12N15/62 C12N15/10 C12N9/16
C12N1/19 C12N1/21 C07K14/315 C07K16/12 A61K31/70
A61K39/09 G01N33/53 G01N33/68 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	DATABASE TREMBL E.M.B.L. Databases Accession Number: Q54914, 1 November 1996 (1996-11-01) PODBIELSKI A ET AL: "ORF 1 AND ORF2 5' REGION" XP002133342 97.2% identity in 141 aa overlap with SeqIdNo.12 abstract --- -/--	3,4

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Date of the actual completion of the international search	Date of mailing of the international search report
17 March 2000	11. 04. 00

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	LARSSON C ET AL: "Experimental vaccination against group B streptococcus, an encapsulated bacterium, with highly purified preparations of cell surface proteins Rib and alpha" INFECT. IMMUN., vol. 64, no. 9, September 1996 (1996-09), pages 3518-3523, XP002125783 cited in the application ---	
A	WO 95 06732 A (MASURE H ROBERT ;TUOMANEN ELAINE (US); PEARCE BARBARA J (US); UNIV) 9 March 1995 (1995-03-09) ---	
A	DATABASE SWISSPROT E.M.B.L. Databases Accession Number: P42422, 1 November 1995 (1995-11-01) YOSHIDA K ET AL: "Hypothetical sensor-like Histidine Kinase in IDH 3' region" XP002133344 30.6% identity in 320 aa overlap with SeqIdNo.20 abstract ---	
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE TREMBL E.M.B.L. databases Accession Number: P94374, 1 May 1997 (1997-05-01) YOSHIDA K ET AL: "HOMOLOGOUS TO MANY ATP-BINDING TRANSPORT PROTEINS" XP002133346 30.2% identity in 235 aa overlap with SeqIdNo.82 abstract ---	
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A	WO 98 18931 A (DOUGHERTY BRIAN A ;HUMAN GENOME SCIENCES INC (US); ROSEN CRAIG A () 7 May 1998 (1998-05-07) -& DATABASE GENESEQ E.M.B.L. Databases Accession Number: V52187, 7 May 1998 (1998-05-07) BARASH S ET AL: "Streptococcus pneumoniae genome fragment SEQ ID NO:54" XP002133371 61.8% identity in 2138 bp overlap with SeqIdNo.124 abstract ---	
T	WO 99 42588 A (BIOCHEM VACCINS INC ;BRODEUR BERNARD R (CA); CHARLEBOIS ISABELLE () 26 August 1999 (1999-08-26) -----	

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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

   1-18 and 23 (all partially) as relating to inventions 1, 6, 10, 13, 35, 41, 62, 63 and 67

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Invention 1: claims 1-18 and 23 (all partially)
A Group B Streptococcus protein having a sequence as depicted in SeqIdNo.2, a fragment, derivative or variant of said protein; a nucleic acid molecule comprising or consisting of SeqIdNo.1, a nucleic acid molecule complementary to said sequence, a nucleic acid molecule encoding for the a derivative or fragment of said protein; a vector comprising said nucleic acid molecule and afferent recombinant DNA practices; an antibody to said protein; an immunogenic composition comprising said protein or said nucleic acid and applications thereof; a method or kit of detection of Group B Streptococcus comprising said protein, said antibody, or said nucleic acid molecule; a method of determining whether said protein represents a potential antimicrobial target which comprises inactivating said protein and determining whether Group B Streptococcus is still viable.

2. Inventions 2-69: claims 1-18 and 23 (all partially)
Idem as subject 1 but limited to each of the polynucleotide and polypeptide sequences as depicted in SeqIdNo:3-137, wherein invention 2 is limited to SeqIdNo:3 and SeqIdNo:4, invention 3 is limited to SeqIdNo:5 and SeqIdNo:6, ..., invention 58 is limited to SeqIdNo:115, ..., and invention 69 is limited to SeqIdNo:136 and 137.

3. Inventions 70: claims 19-22 (all totally)
A method for screening for DNA encoding bacterial cell enveloppe associated or surface antigens in gram positive bacteria comprising a reporter vector including the nucleotide sequence encoding the mature form of the staphylococcus nuclease gene and an upstream promoter region with DNA from a gram positive bacterium; said method wherein the reporter vector is one of the pTREP1-nuc vectors; said method wherein the gram positive bacterium is Group B Streptococcus, Streptococcus pneumoniae, Staphylococcus aureus or pathogenic group A streptococci; said vector which is one of the pTREP1-nuc vectors

For the sake of conciseness, the first and 70th subject-matters are explicitely defined, the other subject-matters are defined by analogy to the subject-matter of invention 1.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/02444

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